Thesis for doctoral degree (Ph.D.)
2017

HAEMOSTATIC AND INFLAMMATORY ALTERATIONS IN HYPERTENSION AND HYPERLIPIDAEMIA, AND THE IMPACT OF ANGIOTENSIN II

Mikael Ekholm

Karolinska Institutet

From THE DEPARTMENT OF CLINICAL SCIENCES, DANDERYD HOSPITAL, DIVISION OF CARDIOVASCULAR MEDICINE

Karolinska Institutet, Stockholm, Sweden

HAEMOSTATIC AND INFLAMMATORY ALTERATIONS IN HYPERTENSION AND HYPERLIPIDAEMIA, AND THE IMPACT OF ANGIOTENSIN II

Mikael Ekholm

Karolinska Institutet

Stockholm 2017

Published by Karolinska Institutet
Printed by E-print AB 2017

ISBN 978-91-7676-784-9

HAEMOSTATIC AND INFLAMMATORY ALTERATIONS IN HYPERTENSION AND HYPERLIPIDAEMIA, AND THE IMPACT OF ANGIOTENSIN II

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Mikael Ekholm, MD

Principal Supervisor:
Professor Thomas Kahan
Karolinska Institutet
Department of Clinical Sciences,
Danderyd Hospital
Division of Cardiovascular Medicine
Stockholm, Sweden

Co-supervisor:
Professor Håkan Wallén
Karolinska Institutet
Department of Clinical Sciences,
Danderyd Hospital
Division of Cardiovascular Medicine
Stockholm, Sweden

Opponent:
Professor Kurt Boman
Umeå University
Department of Public Health and Clinical Medicine
Umeå, Sweden

Examination Board:
Professor Ulf Hedin
Karolinska Institutet
Department of Molecular Medicine and Surgery
Stockholm, Sweden

Professor Toste Länne
Linköping University
Department of Medical and Health Sciences
Division of Cardiovascular Medicine
Linköping, Sweden

Associate professor Gerd Lärfars
Karolinska Institutet
Department of Clinical Science and Education,
Södersjukhuset
Stockholm, Sweden

“To be is to do” – Socrates

“To do is to be” – Jean-Paul Sartre

“Do be do be do” – Frank Sinatra

ABSTRACT

The process of atherosclerosis is multifactorial, and endothelial dysfunction is considered to precede atherosclerosis. Angiotensin (Ang) II, the main effector of renin-angiotensin-aldosterone system (RAAS), is implicated in hypertension and has been shown to promote atherosclerosis.

Familial combined hyperlipidaemia (FCHL) and familial hypercholesterolemia (FH) have been identified as risk factors for increased risk of cardiovascular heart disease and premature death. FCHL has a different phenotype compared to FH, but both lipid disorders are accompanied by subclinical atherosclerosis and endothelial dysfunction. We speculated that patients with hypertension and hyperlipidaemia were more sensitive to the potential proinflammatory and procoagulatory effects of Ang II than healthy individuals. The present research program was set up to investigate the extent to which the RAAS affects the inflammatory and thrombotic properties of individuals with hypertension and hyperlipidaemia.

In **Paper I** we examined the impact of treatment with the ACE inhibitor ramipril on coagulation in patients with mild-to-moderate hypertension. We observed that ramipril attenuates thrombin generation in essential hypertension by reducing thrombin-antithrombin complex, and tended to reduce fibrinogen levels.

In **Paper II** we wanted to clarify the impact of antihypertensive treatment per se. Therefore, we examined the effects of long-term treatment of ramipril compared to the alpha 1-adrenoceptor blocker doxazosin on inflammation and haemostasis in patients with mild-to-moderate hypertension. We found that antihypertensive treatment seems to exert a minor impact on systemic inflammation. Treatment with ramipril, but not doxazosin, appeared to reduce thrombin generation. This extended our previous findings in **paper I** suggesting that treatment with ramipril reduces thrombin generation in addition to the effects on blood pressure reduction alone. Drugs blocking the renin-angiotensin-aldosterone system may reduce atherothrombotic complications beyond their effects of lowering blood pressure. We also observed a decrease in t-PA antigen and a tendency to decreased PAI-1 activity in the doxazosin treated group, which would implicate beneficial effects by treatment with doxazosin in hypertensive patients regarding fibrinolysis. This may be of benefit in the treatment of patients with hypofibrinolysis, such as patients with FCHL.

In **Paper III** we examined how an intravenous infusion of Ang II affected inflammation and haemostasis in patients with FCHL and healthy control subjects. In **Paper IV** we characterized the studied the patients with FCHL, in **paper III**, with respect to insulin resistance and in more detail regarding fibrinolysis. We also performed placebo experiments to make it possible to assess the influence of diurnal variations and to verify the stability of the experimental design. We found that FCHL had an increased systolic blood pressure response during infusion of Ang II compared to controls, indicating an increased vascular

responsiveness in FCHL. Patients with FCHL exhibited a low-grade chronic inflammation, an impaired fibrinolysis, while the coagulation system seemed intact. FCHL shared several characteristics with the metabolic syndrome, including high triglyceride and low HDL cholesterol levels, insulin resistance and high body mass index. An infusion of Ang II increased systemic inflammation in a similar way in FCHL and controls. Ang II did not have any impact on thrombin generation, in either FCHL or controls. Ang II did not affect fibrinolysis in FCHL, whereas fibrinolysis was enhanced in healthy controls. The different responses to Ang II stimulation probably involved t-PA activity but not PAI-1 activity, and this suggests that patients with FCHL were incapable of increasing fibrinolysis in response to Ang II. We could not observe any short-term effects on PAI-1 activity, in either FCHL or controls. Our findings suggested that patients with FCHL had a low-grade chronic inflammation, impaired fibrinolysis and insulin resistance, contributing to the risk of cardiovascular heart disease and premature death in FCHL. We also suggested that Ang II acted as a proinflammatory and enhanced fibrinolysis, without impact on thrombin generation. However, taking the possible effects of diurnal variations of our coagulation markers, not taken into account in paper III, and analysing the impact of Ang II during the *ongoing* infusion time, post hoc analyses showed that thrombin generation instead increased, similarly in FCHL and controls. Hence, our new conclusion became that Ang II acts as a prothrombotic agent.

In **Paper V** we examined how an intravenous infusion of Ang II affected inflammation and haemostasis in patients with FH and healthy controls. We also performed placebo experiments to make it possible to assess the influence of diurnal variations and to verify the stability of the experimental design. We found that patients with FH had higher systolic blood pressure than controls at baseline, whereas blood pressure responses were equal in FH and controls. FH showed an intact fibrinolysis and an increased thrombin generation potential compared to controls, but did not show any convincing signs of an on-going low-grade inflammation. A systemic infusion of Ang II caused an increase in systemic inflammation, fibrinolysis and possibly also thrombin generation similar in FH and control subjects. During Ang II infusion FH exhibited possible signs of an activated anticoagulant system. Our findings suggested that patients with FH had an affected coagulation system, rather than altered fibrinolysis or inflammation, contributing to the increased risk of cardiovascular heart disease and premature death in FH.

Thus, blocking the renin-angiotensin-aldosterone system by an ACE inhibitor may prevent atherothrombotic complications in hypertensive patients beyond the effects on BP by reducing thrombin formation. Different mechanisms may contribute to the increased incidence of cardiovascular complications in patients with FCHL and FH. A beneficial effect of ACE inhibition in patients with FCHL might be to attenuate inflammation in combination with its documented positive influence on insulin resistance, while in patients with FH, may benefit be obtained mainly by reduced thrombin generation.

LIST OF SCIENTIFIC PAPERS

I. **Mikael Ekholm,** N Håkan Wallén, Hans Johnsson, Keith Eliasson, Thomas Kahan. Long-term angiotensin-converting enzyme inhibition with ramipril reduces thrombin generation in human hypertension. *Clin Sci (Lond). 2002; 103(2):151-5.*

II. **Mikael Ekholm,** Andreas Jekell, N Håkan Wallén, Bruna Gigante, Thomas Kahan. The effects of angiotensin converting enzyme inhibition and alpha 1-adrenergic receptor blockade on inflammation and hemostasis in human hypertension. *Submitted*.

III. **Mikael Ekholm,** Thomas Kahan, Gun Jörneskog, Anders Bröijersen, N Håkan Wallén. Angiotensin II infusion in man is proinflammatory but has no short-term effects on thrombin generation in vivo. *Thromb Res. 2009; 124(1):110-5.*

IV. **Mikael Ekholm,** Thomas Kahan, Gun Jörneskog, Anders Bröijersen, N Håkan Wallén. Infusion of angiotensin II increases fibrinolysis in healthy subjects but not in familial combined hyperlipidaemia. *Blood Coagul Fibrinolysis. 2016;27(1):113-6.*

V. **Mikael Ekholm,** Thomas Kahan, Gun Jörneskog, Jonas Brinck, N.Håkan Wallén. Haemostaticatic and inflammatory alterations in familial hypercholesterolemia, and the impact of angiotensin II infusion. *J Renin Angiotensin Aldosterone Syst. 2015;16(2):328-38.*

The articles will be referred to in the text as **Papers I-V** and are reproduced in full as appendices.

CONTENTS

LIST OF ABBREVIATIONS

ACE	angiotensin converting enzyme
AKT	ak strain transforming or protein kinase B
Ang	angiotensin
ANOVA	analysis of variance
APC	activated protein C
ARB	angiotensin receptor blocker
AT	antithrombin
AT1R	angiotensin 1 receptor
BP	blood pressure
CAT	calibrated automated thrombogram
CCB	calcium channel blocker
CD	cluster of differentiation
CHD	coronary heart disease
COX	cyclooxygenase
CRP	c-reactive protein
CVD	cardiovascular disease
ECM	extracellular matrix
ED	endothelial dysfunction
EDCF	endothelium-contracting factor
EDRF	endothelium-derived relaxing factor
EPCR	endothelial protein C receptor
ERK	extracellular signal regulated kinase
F	coagulation factor or clotting factor
F1+2	prothrombin fragment 1+2
FCHL	familial combined hyperlipidaemia
FH	familial hypercholesterolemia
GAG	glycosaminoglycan
GLUT-4	glucose transporter type-4
GP	glycoprotein
GPER	G protein oestrogen receptor
HDL	high-density lipoprotein
HOMA-IR	homeostasis model assessment of insulin resistance
Hs	high sensitive
ICAM	intracellular adhesion molecule
IDL	intermediate-density lipoprotein
IL	interleukin
LDL	low-density lipoprotein
LDLR	low-density lipoprotein receptor
Lox	lectin-like oxidized LDL receptor
MAC-1	macrophage antigen-1

MAP	mitogen-activated protein
MCP-1	monocyte chemoattractant protein-1
MR	mineralocorticoid receptor
MP	microparticle
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NF-κβ	nuclear factor kappa -light-chain-enhancer of activated B-cells
NO	nitric oxide
NOS	nitric oxide synthase
Nox	NADPH oxidase
Ox	oxidized
PAF	platelet activating factor
PAI-1	plasminogen activator inhibitor-1
PAP	plasmin-antiplasmin
PAR	protease-activated receptor
PGI2	prostaglandin I2, or prostacyclin
PSGL-1	platelet-selectin glycoprotein ligand-1
RAAS	renin-angiotensin-aldosterone system
RANTES	regulated on activation, normal T cell expressed and secreted
Redox	reduction-oxidation reaction
ROS	reactive oxygen species
SD	standard deviation
TAFI	thrombin activatable fibrinolysis inhibitor
TAT	thrombin-antithrombin
TF	tissue factor, or thromboplastin
TFPI	tissue factor pathway inhibitor
t-PA	tissue plasminogen activator
TXA2	thromboxane A2
VIIa	activated factor VII
VCAM	vascular cell adhesion molecule
VLDL	very-low density lipoprotein
vWF	von Willebrand factor

1 INTRODUCTION

1.1 GENERAL BACKGROUND

The most common cause of death in economically developed countries is cardiovascular disease (CVD). During the period 1990 to 2010, deaths from CVD increased from 26% to 30% of all deaths globally. Death rate caused by CVD is expected to decrease to 24% of all deaths in 2030, but will still retain its leading position due to an increasing prevalence in developing countries (1). The expected decline globally is thought to be due to a dramatic shift in deaths from infectious diseases and malnutrition, with very short life expectancy, compared to CVD and cancer. Given the high mortality and morbidity burden in CVD, it is of great importance for societies and their health care systems to improve strategies to decrease the incidence of CVD in the future (2).

A well-known cause of death within CVD is coronary heart disease (CHD), and traditional risk factors for this disease are hypertension, hypercholesterolemia, diabetes mellitus, obesity, tobacco smoking, age, male sex and family history. In the EUROASPIRE III trial (figure 1), almost 9000 participants with CHD in 22 European countries were included. The study showed that more than 50% of the patients had hypertension or hypercholesterolemia and 35% had diabetes mellitus or were obese (3).

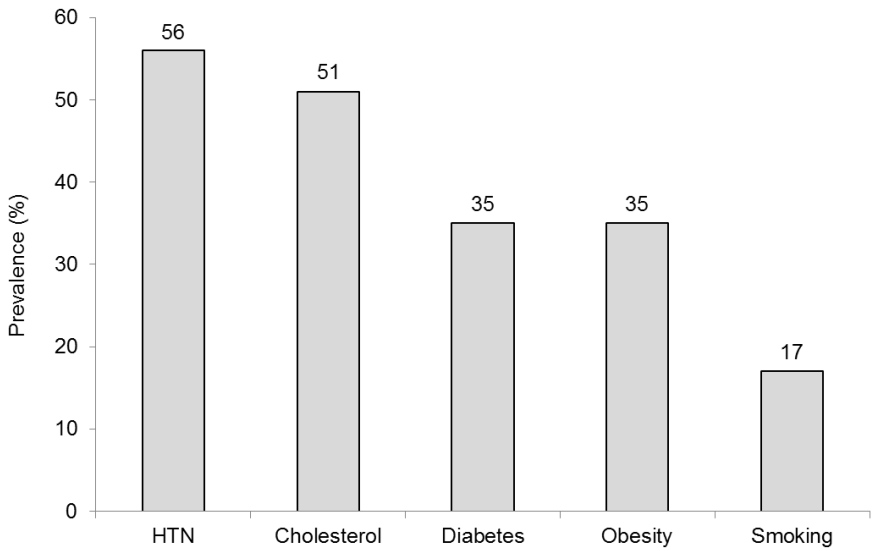

Figure 1. The EUROASPIRE III survey. The bars show prevalence of cardiovascular disease risk factors in subjects with established coronary heart disease (n = 8966). Hypertension was characterized as a blood pressure above 140/90 mmHg. High cholesterol levels were characterized as total cholesterol above 4.5 mmol/L and obesity was characterized as a body mass index above 30 kg/m2. HTN, hypertension. Figure modified from Volpe M, 2012 (4).

The INTERHEART study (5) studied incidence of acute myocardial infarction globally. This large-scale study enrolled around 15 000 cases and 15 000 controls. The association of nine modifiable risk factors to acute myocardial infarction and a risk referred to as the population attributable risk were calculated. The study showed that the incidence of an acute myocardial infarction was in more than 90% of the cases associated with the nine measured risk factors. The most important risk factor turned out to be abnormal lipids, apolipoprotein (Apo) B/ApoA1 ratio, in all geographic regions. Five of the traditionally most common and well-described risk factors, hyperlipidaemia, hypertension, tobacco smoking, diabetes mellitus and obesity, accounted for around 80% of the population attributable risk.

Several additional risk factors for CVD have been recognized: hypercoagulability, impaired fibrinolysis, hyperinsulinemia, physical inactivity, impaired high-density lipoprotein (HDL) cholesterol and psychosocial factors. Most of the risk factors are related to lifestyle and metabolic disorders, and often several of the risk factors are present in a cluster. The most common condition with several risk factors is the metabolic syndrome. Varying definitions are given by different organizations, but the most common risk factors referred to include hypertension, dyslipidaemia, insulin resistance and abdominal obesity, culminating in an elevated risk of CVD and type 2 diabetes mellitus (6).

Angiotensin (Ang) II has an important role for inflammation in the vessels. During the past decades Ang II has been shown to initiate and accelerate hypertension, endothelial dysfunction (ED) and atherosclerosis (7). Conversely, inhibition of the renin-angiotensin-aldosterone system (RAAS) reduces atherosclerosis in animal models (8), and death from CVD in humans (9).

Ang II may have an impact on thrombosis. The extrinsic coagulation pathway is of great importance in the initiation of blood coagulation, and tissue factor (TF, also known as thromboplastin or clotting factor (F) III) initiates this pathway. Ang II may increase TF expression in monocytes, endothelial cells (EC) and in vascular smooth muscle cells (VSMC) (10, 11), and conversely treatment with RAAS blockade can diminish TF in plasma and in monocytes (12). We have reported that infusion with Ang II induces thrombocyte activation acutely (13). Also platelets have been shown to possess receptors for Ang II, angiotensin type 1 receptor (AT1R) subtype, but the cinical importance of these receptors is not known. TF is accepted as the initiator of coagulation, and the amount of TF exposure will predict whether or not clotting will occur. It has been assumed that an increased expression of TF is an adaptive defence mechanism that aims to facilitate haemostasis at sites of injury, but these mechanisms can contribute to a prothrombotic state in a number of diseases.

Accumulating evidence indicates an association between hypercholesterolemia and activation of the RAAS in the progress of atherosclerosis (14). Exposure of Ang II to hypercholesterolemic animals has been reported to be potently proatherogenic, and data strongly suggest that Ang II potentiates atherosclerosis in experimental models of hyperlipidaemia (7, 15). Conversely, it seems that ACE inhibitors offer vasoprotective effects

by reducing atherosclerosis, and this is well documented in animal experiments (16-18) and in large human trials (9, 19, 20). In addition, studies have shown that Ang II has important effects with regard to oxidation of low-density lipoprotein (LDL) cholesterol in the vessel wall (16), and on macrophage uptake of oxidized (ox) LDL cholesterol and entry into vessels (21).

A common primary lipid disorder in humans is FCHL (phenotype IIb according to the Fredrickson classification) (22), defined by an elevation of triglycerides and cholesterol in combination with reduced plasma HDL cholesterol. Patients with FCHL also exhibit elevated very low density lipoprotein (VLDL) cholesterol and small dense LDL cholesterol. FCHL has an association with insulin resistance, impaired endothelial reactivity, hypercoagulability, impaired fibrinolysis and systemic inflammation (23-27). Familial hypercholesterolemia (FH) (phenotype IIa according to the Fredrickson classification) (22) is less common and is characterized mainly by elevated total and LDL cholesterol. FH exhibits impaired endothelial reactivity (28) and a prothrombotic state without influence on fibrinolysis (29, 30). Together, FCHL and FH phenotypes account for about half of the primary lipid disorders.

The present research program was set up to investigate whether activation of RAAS in hypertensive patients has an impact on inflammation and haemostasis. We also wanted to clarify if the potential effects blocking the RAAS on inflammation and haemostasis were due to the antihypertensive effect per se. Patients with FCHL have a different phenotype as compared to FH. Both conditions have a poor vascular outcome and are recognized by impaired endothelial reactivity, but otherwise different characteristics regarding inflammation and haemostasis. We therefore studied the inflammatory and haemostatic responses to Ang II stimulation in these subjects separately.

1.2 INFLAMMATION IN VESSELS

Atherosclerosis is nowadays considered a disease caused by a chronic inflammation, associated with ED (31). Low-grade inflammation contributes to atherosclerosis, and several mediators of inflammation are up-regulated in subjects with atherosclerotic disease (31). Among the markers of inflammation for diagnostic use, the cytokines interleukin (IL)-6, and in particular C-reactive protein (CRP), have generated considerable attention. CRP is generated by hepatic cells and is modulated by IL-6, but also by the cytokines tumor necrosis factor (TNF)-α and IL-1 (32), thereby contributing to the up-regulation of monocyte chemoattractant protein-1 (MCP-1) and selectins, such as P- and E-selectins and cell adhesion molecules, such as intracellular adhesion molecule -1 (ICAM-1) and cell adhesion molecule-1 (VCAM-1). CRP attenuates the synthesis of endothelial nitric oxide (NO) (33), and causes augmented plasminogen activator inhibitor-1 (PAI-1) (34). Increased concentrations of acute phase reactants like CRP, IL-6, leukocyte count and fibrinogen are all associated with an increased risk of CVD (35-37). Phospholipase A2, which is implicated in the oxidation of LDL and subsequent oxidative stress and inflammation, can

predict atherosclerotic disease (38). These data clearly support a pivotal role for cytokines in the inflammatory process in early stages of atherogenesis.

Inflammatory mediators have been associated with components of metabolic disturbances, and cytokines might be a link between dysregulated metabolism and inflammation, as CRP, fibrinogen and IL-6 are closely related to the metabolic syndrome (32).

1.3 ENDOTHELIAL DYSFUNCTION AND ATHEROSCLEROSIS

The endothelium has an important function in preserving a physiological structure and function. Healthy, intact endothelium exhibits a thromboresistant, protective surface between the vascular lumen and VSMCs in the vessel wall, with the lamina elastic interna in between. In particular, a normally functioning endothelium prevents platelet adhesion (39). ECs form a monolayer that produces factors that regulate vascular tone, inflammation, haemostasis, vascular cell growth and death, angiogenesis and the migration of leukocytes. Vascular tone is dependent on a delicate balance between vascular dilators, such as NO, and vascular constrictors, such as Ang II. Also, VSMCs are affected by ECs and other factors, and VSMCs can themselves release cytokines and growth-regulatory mediators, which in turn have an impact on vessel phenotype and growth.

1.3.1 Oxidative stress

In 1985, Sies described oxidative stress as an imbalance between anti- and prooxidants, with a subsequent increase of reactive oxygen species (ROS) bioavailability, leading to tissue damage (40). An important factor of the biology of ECs is the cell reduction-oxidation reaction (redox) state. A molecule of particular importance in endothelial function is NO. The traditional risk factors for CVD can initiate ED by changing the cell redox state and, consequently, the oxidative stress in the vascular wall.

The ratio between ROS and NO regulates the redox state and is of vital importance for proper function of the vascular endothelium. Increased generation of superoxide anion, •O2 and, subsequently, oxidative stress result in an enhanced catabolism of NO, which leads to ED and impaired vasodilatation. ROS also has the ability to reduce the activity of NO synthase (NOS) and to increase the breakdown of NO. Also, NO is a potent endogenous inhibitor of VSMC migration and growth (41) and impairs the up-regulation of adhesion molecules and cytokines (42). The transcription of the pleiotropic nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) has a pivotal role in endothelial up-regulation of cytokines and adhesion molecules, and NO is a powerful inhibitor of activation of NF-κB (43). It is to be noted that NO and the superoxide anion react to form the powerful oxidant peroxynitrite, ONOO-, that can damage ECs. ROS also lowers the availability of tetrahydrobiopterin. If the latter occurs, the oxygenase function of NOS is replaced by its reductase function and ROS are produced instead of NO, which increases NF-κB activity, and the expression of cytokines (42). An imbalance between NO and ROS increases the risk for vasospasm, VSMC proliferation, proinflammatory and prooxidant states and ED

can create an imbalance between tissue plasminogen activator (t-PA) and PAI-1 and may predispose a prothrombotic state.

Oxidative stress up-regulates and activates redox-sensitive genes for chemokines (such as MCP-1) and vascular adhesion molecules (such as VCAM-1 and ICAM-1). The superoxide anion may mediate increased activity of NF-κB that has a vital function in up-regulating these proinflammatory genes (44). As a consequence of this activity, leukocytes interact with the endothelium, and subsequently transmigrate into the subendothelial layer of the vessel wall. After transmigration of the leukocytes, cytokines (such as IL-6) are released, resulting in recruitment of additional monocytes. In the vessel wall, monocytes subsequently differentiate into macrophages. Via the scavenger receptors, macrophages take up oxLDL and are then transformed into foam cells, present in the early stages of atherosclerosis. As the plaque progresses at inflammatory sites, macrophages and other migrating cells, as well as activated ECs, produce cytokines and matrix metalloproteinases, eventually causing the plaque to rupture.

1.3.2 Recruitment of leukocytes, platelet dependent

ED may be defined as an imbalance between, on the one hand vasodilating, and on the other hand vasoconstricting substances, produced by the endothelium (45). Current concepts of atherogenesis include involvement of platelets, the immune system and chronic inflammation (46).

The healthy endothelium controls platelet activity through inhibitory mechanisms, while a systemic inflammatory environment induces ECs to develop a phenotype that make them adhesive for platelets (47). Numerous studies have shown that platelets have the ability to adhere to the intact ECs and also to modulate their function. Thus, exposure of the subendothelial surface, as in plaque rupture, is not necessary for platelet adhesion to vascular cells. Activated platelets release mediators and growth hormones, which will induce up-regulation of adhesion molecules and the release of chemoattractants that, in turn, regulate the adhesion and subsequent transmigration of leukocytes into the vessel wall. It is important to take into account when analysing platelets in animal models that human platelets differ from platelets in for example the mouse in many important ways (such as difference in expression of surface receptors, higher platelet count), and the results in animal experiments can not be uncritically applied to the human situation (39).

The contact between platelets and the inflamed ECs is accomplished by the binding of endothelial P-selectin to glycoprotein (GP) Ib/IX/V (also referred to as the von Willebrand factor (vWF) receptor complex). Platelet-selectin glycoprotein ligand-1 (PSGL-1), interacting with P-selectin, present on leukocytes and to a minor degree also on platelets, mediates rolling of platelets to ECs under high shear stress. However, the association between PSGL-1/GPIb/IX/V and P-selectin is insufficient for a stable and durable adhesion. The firm and long-lasting binding of platelets to ECs is mediated through the β_3 integrins αIIbβ3 (GPIIbIIIa) and αvβ3, the vitronectin receptor.

Adherent platelets undergo a series of alterations, both morphological and biochemical, leading to release of potent proinflammatory and mitogenic substances, such as IL-1β, IL-8, cluster of differentiation (CD) 40L, platelet factor 4, thromboxane A2 (TXA2), platelet-derived growth factor, platelet activating factor (PAF) and regulated on activation, normal T cell expressed and secreted (RANTES), thereby changing the phenotype of the ECs into a chemotactic, adhesive, and proteolytic state. The changes of the ECs, induced by platelets, will support chemotaxis, and subsequently adhesion of monocytes to the inflammatiory sites (48). Activated platelets also secrete glycosaminoglycans (GAG)s that contribute to the immobilization of chemokines and the adhesion and transmigration of leukocytes (49).

The firm adhesion of neutropils to platelets is facilitated by macrophage antigen-1 (MAC-1 or αMβ2) activation, induced by P-selectin/ PSGL-1 and augmented by platelet chemokines, arachidonic acid metabolites and inflammatory lipids. The interaction between MAC-1 and platelet surface ligands (ICAM-2), GPIb/IX/V and αIIbβ3-bound fibrinogen) also elicits signalling to promote leukocyte activation and migration through the endothelium and extravasation (50). Binding of fibrinogen to MAC-1 also primes leukocyte release of the cytokines (IL-1β, IL-8, IL-6 and TNF-α), which potentiates the proinflammatory response. The binding of fibrinogen and fibrin on mononuclear cells are mediated by Toll-like receptor 4 (51). Lymphocyte function-associated antigen 1, LFA-1 or αLβ2, ligation with ICAM-2 is also present, but MAC-1 has been shown to have the dominant role in promoting stable leukocyte-platelet interaction. Figure 2 illustrates platelet dependent recruitment of leukocytes.

1.3.3 Recruitment of leukocytes, platelet independent

During inflammation, ECs, leukocytes and platelets may release a variety of cytokines and chemokines. In the leukocyte adhesion cascade, leukocytes first roll on inflamed ECs. Rolling is initiated and mediated by the interaction of endothelial P- or E-selectin with their counterparts PSGL-1 and E-selectin ligand, respectively. Conversely, P- and E-selectins are not expressed at the cell surface in absence of inflammatory stimuli. The P-selectin molecule is stored in platelet α-granules (52), or in EC Weibel-Palade bodies, and may be released upon stimulation (53).

The firm attachment of circulating cells to the inflamed vasculature is mediated by the leukocytes-expressed β2-integrins. The most powerful activators of these integrins are chemokines, secreted by cytokine-activated ECs (54), stromal cells, platelets (55), or by leukocytes themselves. PAF is a chemoattractant proinflammatory lipid, which acts in cooperation with P-selectin to cause integrin activation (56). In blood vessels, chemokines are sequestrated by GAGs on the luminal surface of inflamed ECs to be ideally exposed to leukocytes (57).

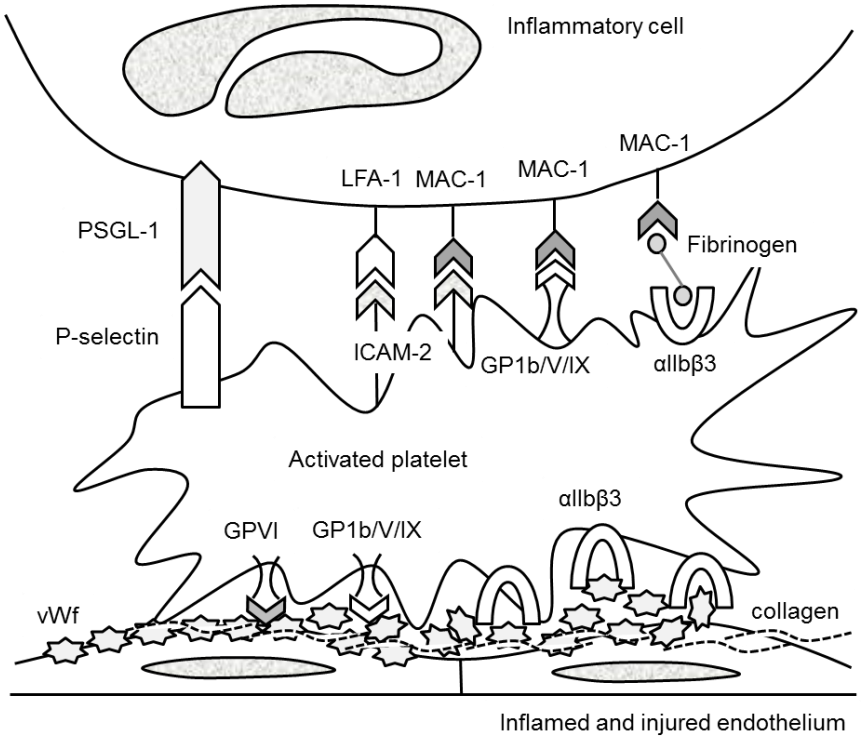

Figure 2. Recruitment of leukocytes, platelet dependent. Leukocytes can be recruited to inflamed endothelial cells through interaction with platelets. The first contact by rolling of platelets on endothelial cells is mediated by interaction of endothelial P-selectin to platelet PSGL-1 or GPIb/IX/V, respectively. Firm binding is then mediated through the β3 integrins. Adherent platelets release inflammatory substances, which support chemotaxis and adhesion of monocytes. The β2 integrin, MAC-1 have a dominant role in promoting stable leukocyte-platelet interaction. GP, glycoprotein; ICAM, intracellular cell adhesion molecule; LFA, lymphocyte function-associated antigen; PSGL, platelet-selectin glycoprotein ligand; MAC, macrophage antigen; vWF, von Willebrand factor; αvβ3, the receptor for vitronectin and αIIbβ3, also called GP IIbIIIa. Figure modified from Ghasemzadeh M, 2012 (58).

Following integrin priming by selectins, and subsequently slow rolling, chemokines on neutrophils induce a rapid activation of integrins (59). The key role of the β2-integrins is well established. Leukocyte adhesion and transmigration at inflammatory sites is dependent on the β2-integrin ligation of MAC-1 and LFA-1 on leukocytes, with their specific ECs ligands, ICAM-1 and ICAM-2 (60). Also, the β1-integrin very late antigen 4, VLA-4 or α4β1, and its major endothelial counter-receptor VCAM-1, have an important role in monocyte and lymphocyte arrest. Figure 3 illustrates platelet independent recruitment of leukocytes.

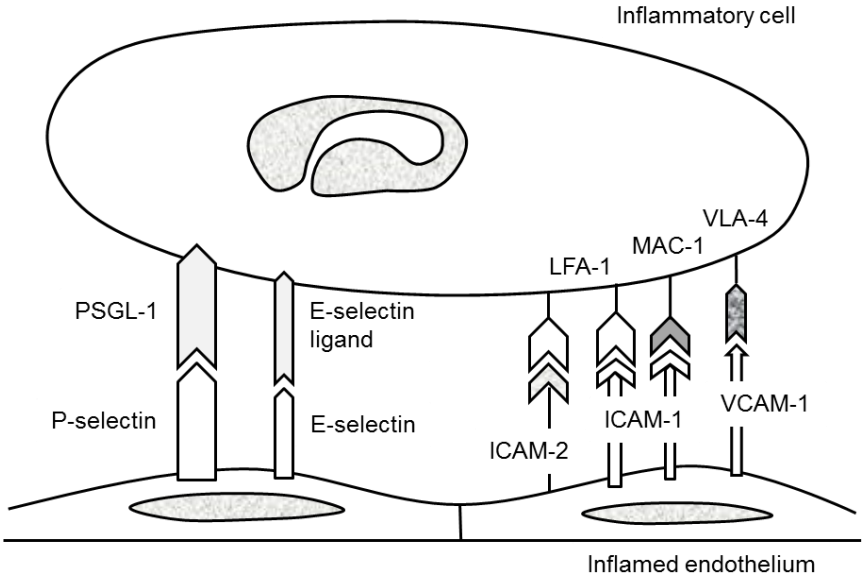

Figure 3. Recruitment of leukocytes, platelet independent. Leukocytes can adhere to inflamed endothelial cells through specific interaction with endothelial cells. At first, rolling is initiated by binding of selectins with their receptors, while firm attachment is mediated by the leukocytes-expressed MAC-1 and LFA-1 (β2-integrins) and by VLA-4 (a β1-integrin). VCAM, vascular cellular adhesion molecule; ICAM, intracellular cell adhesion molecule; LFA, lymphocyte function-associated antigen; MAC, macrophage antigen; PSGL, platelet-selectin glycoprotein ligand and VLA, very late antigen. Figure modified from Ghasemzadeh M, 2012 (58).

1.4 THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

The RAAS is a dynamic physiologic system and is central in regulating the balance of fluids and electrolytes and blood pressure (BP). All RAAS peptides are derived from angiotensinogen. Renin, the rate-limiting enzyme is produced in the kidney (juxtaglomerular cells), and is released in response to vasodilation or low sodium. Renin cleaves angiotensinogen into Ang I. ACE hereafter cleaves Ang I to generate Ang II, which is the predominant peptide of the RAAS (61).

Ang II primarily exerts its influence through the receptors AT1R and AT2R. A number of signalling pathways are activated when Ang II interacts with AT1R and AT2R. The main AT1R-mediated Ang II effects include vasoconstriction by VSMC stimulation, sodium retention in the kidneys, and aldosterone release from the adrenal cortex (62). AT2R-mediated effects generally oppose those effects mediated by AT1R, and include vasodilatation and anti-inflammatory effects in VSMCs, but also anti-proliferative effects in the myocardium (63). Figure 4 summarizes the different RAAS components.

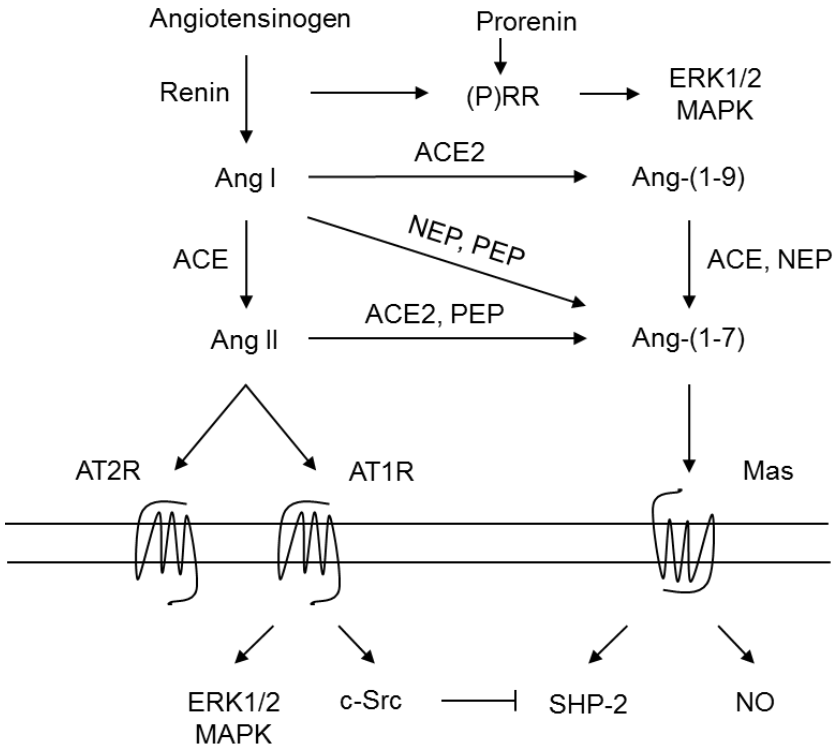

Figure 4. The renin-angiotensin-aldosterone system. Renin is secreted due to various stimuli, and then cleaves angiotensinogen into the inactive decapeptide Ang I. Renin and prorenin can also interact with (pro) renin receptors to activate the MAP kinases ERK1/2 and p38 pathways. ACE cleaves Ang I into the octapeptide Ang II, or to Ang-(1-7) by ACE2 and probably ACE. ACE2 may also produce Ang-(1-7) from Ang II. ACE also inactivates bradykinin into inactive fragments. Importantly, Ang II may be generated directly from angiotensinogen through non-ACE pathways. Ang II activates AT1R, a G protein-coupled receptor. Vasoconstriction and stimulation of aldosterone tend to elevate blood pressure. Ang II also activates AT2R, a G protein-coupled receptor, which can antagonize the effects of activation of the AT1R. Ang, angiotensin; ACE, angiotensin converting enzyme; MAPK, mitogen-activated protein kinase; extracellular signal regulated kinase, ERK; angiotensin 1 type receptor, AT1R; angiotensin 2 type receptor, AT2R; (P)RR, (pro)renin receptor; c-Src, cellular Src kinase, a non-receptor tyrosine kinase; SHP-2, Src-homology 2 domain-containing phosphatase 2; NO, nitric oxide; NEP, neutral endopeptidase and PEP, prolyl endopeptidase.

1.4.1 The ACE2-Ang-(1-7)-Mas axis

Studies have identified a number of angiotensinogen derived peptides and their receptors (figure 4). Ang-(1-7) is derived from Ang II through the influence of ACE2. Then Ang-(1-7) exerts its effect via the Mas receptor (64). The axis of ACE2-Ang-(1-7)-Mas may lead to vasodilatation via activation of NO, and decreased fibrosis, thereby enhancing the effect of ACE inhibitor blockade of Ang II (65). Ang-(1-7) also mediates anti-inflammatory and

anti-thrombotic effects (66) via activation of NO and inhibition of ROS, derived from nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase (Nox) (67). Increased Ang-(1-7) has been associated with a favourable phenotype, with attenuated inflammation in atherosclerotic plaques (68). In summary, the ACE2-Ang-(1-7)-Mas axis appears to work as a system of opposite effects, that may have the ability to complement the ACE-Ang II-AT1R axis.

1.4.2 Aldosterone

Aldosterone is produced in the adrenal cortex and acts on sodium reabsorption in the kidney. Common stimuli are Ang II, high plasma levels of potassium and adrenocorticotropic hormone. Aldosterone is also implicated in vascular inflammation, oxidative stress, fibrosis, remodelling and ED, particularly in the presence of salt (69). Conversely, vascular remodelling effects are reduced by the use of mineralocorticoid receptor (MR) blockers (70). In VSMCs and in ECs, aldosterone exerts its effects via mitogen-activated protein (MAP) kinase (also known as extracellular signal regulated kinase (ERK)), c-Src (cellular Src kinase, a non-receptor tyrosine kinase), and participates in epidermal growth factor receptor transactivation (71, 72) (figure 5). Aldosterone induced increase in oxidative stress in VSMCs may also have an impact on ED through a reduction in NO bioavailability (72). Vascular inflammation in ECs is promoted by aldosterone induced expression of ICAM-1 and adhesion of leukocytes in an MR dependent manner (73).

There is cross-talk between aldosterone and Ang II in VSMCs. Aldosterone increases the expression of AT1R in vivo (74), and Ang II stimulates aldosterone synthesis by the adrenal gland.

There are synergistic effects between aldosterone and Ang II on VSMC proliferation (75), migration (76), constriction (77) and senescence (78). The synergistic effect of aldosterone and Ang II in VSMC proliferation is a very rapid response, already after 5 minutes. This response is compatible with a non-genomic and MR dependent pathway via activation of AT1R and transactivation of epidermal growth factor receptor. A second peak, between 2 and 4 h, is compatible with a genomic pathway. Aldosterone injections in healthy subjects induce a rapid (within 10 minutes) increase in vascular resistance (79).

The G protein oestrogen receptor (GPER) is the principal mediator of oestrogen effects, but studies have reported that aldosterone is a much more potent agonist at GPER than oestrogen (80). GPER is a widely expressed receptor in cardiovascular tissues, and is present in the heart, in the ECs (81) and in VSMCs. GPER has been shown to mediate vasodilatation and to lower BP, and the vasodilator effects appears to be EC dependent, secondary to activation to phosphatidylinositol 3-kinase and NOS (80). The endothelial dependent vasodilatation seems to be a function of gender, age, or both, with a strong impact in premenopausal women, and a weak influence in men. MR antagonists may also act as GPER antagonists (80).

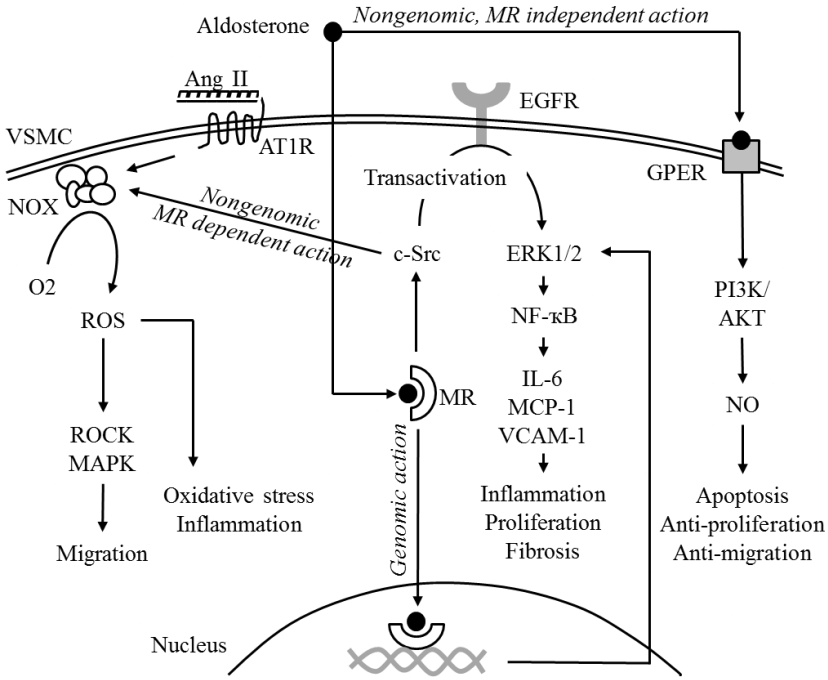

Figure 5. Aldosterone exerts its effects via a non-genomic or a genomic action. Non-genomic pathways are MR dependent or independent. In the MR-dependent pathway aldosterone induces vasoconstrictor effects via a rapid activation of ERK1/2 through transactivation of EGFR and c-Src. C-Src also induces ROS production via NOX, and ROS activates ROCK and MAP kinase. In the genomic pathway aldosterone binds to the MR and translocates to the nucleus of the cell and activates genes, which in turns activates ERK1/2. Aldosterone also have vasodilator effetc via GPER activation and a PI3K dependent increase in NO activity. MR, mineralocorticoid receptor; ERK, extracellular signal-regulated kinase; c-Src, cellular Src kinase, a non-receptor tyrosine kinase; EGFR, epidermal growth factor receptor; ROS, reactive oxygen species; ROCK; Rho-associated protein kinase; NOX, nicotinamide-adenine dinukleotidfosfat oxidase; VSMC, vascular smooth muscle cell; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B-cells; GPER, G-protein-coupled oestrogen receptor (also referred to as GPR30, G protein-coupled receptor 30); PI3K, phosphatidylinositol 3-kinase; AKT, ak strain transforming (also referred to as protein kinase B) and NO, nitric oxide. Figure modified from Briet M, 2013 (82).

The endothelium mediated vasodilation by aldosterone seems to be induced by activation of GPER, whereas the vasoconstrictor actions may be dependent on MR activation in VSMCs. Thus, the balance between *endothelial vasodilator* (GPER-mediated) and *VSMCs vasoconstrictor* effects (MR-dependent) appears to be a pivotal determinant of the effect of aldosterone on regulation of vascular contractility (83).

GPER is also expressed in normal hepatocytes and GPER activation increases LDL receptor expression and suppresses circulating LDL cholesterol, while oestrogen deficiency

increases LDL cholesterol. The clearance of LDL via GPER activation has been linked to down-regulated levels of proprotein convertase subtilisin kexin type 9 (84).

Taken together, GPER seems to influence two of the most important atherosclerotic risk factors, hypertension and dyslipidemia, which implies that GPER may influence the development of atherosclerotic complications.

In vivo data have shown that mutual influence between aldosterone-stimulated MR and Ang receptor pathways plays a role for vascular effects of aldosterone. The AT1R seems to be required for MR-induced ED and vascular remodelling, inflammation and oxidative stress (85). Aldosterone has been linked to the metabolic syndrome and obese patients, and in the Framingham offspring study aldosterone was a predictor for incidence in the metabolic syndrome (86). An association between body weight mass and plasma aldosterone concentration has been shown in normotensive overweight patients. (87). Also, aldosterone decreases after weight loss (88), and aldosterone might be considered a potential adipocyte-derived factor since adipocytes have been shown to synthesize aldosterone in an AT1R dependent manner (89).

1.4.3 Renin, prorenin and renin-prorenin receptor

The RAAS exert effects independent of Ang I and Ang II (figure 4). This includes the direct effect of renin and prorenin, its proenzyme inactive form (90). The use of both ACE inhibitors and AT1R blockers has been reported to result in an at least 3-fold increase in plasma renin activity (91). The (pro) renin receptor may bind both renin and prorenin. The active receptor activates the MAP kinases ERK1/2 and p38 pathways. This entails in turn cell growth and fibrosis in cardiomyocytes, ECs and VSMCs (92). Inhibition of renin with aliskiren (a renin inhibitor) led to reduction in BP, improvements in systemic insulin resistance, improved insulin signalling and glucose uptake (93). These improvements were also associated with decreased levels of Ang II, aldosterone, AT1R, and consequently attenuated oxidative stress and fibrosis. For this reason it is difficult to interpret whether these effects were due to direct renin blockade or through decreasing downstream components of the RAAS. The exact significance of the (pro)renin receptor still is unclear, and a role for this receptor is lacking (94).

1.4.4 Alternative enzymes that generate Ang II

The RAAS system is complicated by enzymes (besides renin and ACE) that can generate Ang II. Chymase, generated in mast cells, is supposed to be the main enzyme responsible for cleaving Ang I into Ang II (95). Ang II can also be formed by direct proteolysis of angiotensinogen by a number of other enzymes, such as cathepsin G, tonin and t-PA, but the contribution of these enzymes is controversial (96, 97). There are estimates that at least 40% of Ang II is formed by non-ACE pathways (98). This suggests that full-scale suppression of the RAAS is not possible by ACE inhibition alone.

1.4.5 Atherosclerosis and the RAAS

The RAAS is of vital importance in the pathobiology of vascular disease, and convincing data indicate that Ang II promotes atherosclerosis (99). Thus, it has been proposed that RAAS inhibition may have anti-atherosclerotic effects beyond the effects of the BP reduction (9).

Ang II causes oxidative stress and impaired NO activity. A cascade of intracellular signalling responses is initiated when Ang II binds to the AT1R. Ang II is a powerful activator of vascular Nox (of which Nox 1, 2, 4 and 5 are present in arteries), that induces the production of ROS (superoxide anion and hydrogen peroxide, H2O2) from ECs and VSMCs (100), and activation of redox-sensitive kinases (101). Activation of the AT1R induces generation of Nox-derived ROS that act as a second messenger to stimulate multiple signalling molecules (102). ROS also increase intracellular calcium and activate NF-κB and activating protein-1. These molecules participate in migration and cell-growth, and also in the expression of inflammatory genes and extracellular matrix (ECM) modulation. Ang II also activates RhoA/Rho-kinase, important in vascular contraction and growth (103).

A pivotal step in atherosclerosis is the attraction of leukocytes to the endothelium. One of the primary Ang II effects is to induce ED and to generate a proinflammatory phenotype in human VSMCs. Ang II activate NF-κB and stimulates the up-regulation of the adhesion molecule VCAM-1, the chemokine MCP-1 and the cytokine IL-6. Also, ICAM-1 and E-selectin may mediate Ang II-induced monocyte adhesion (104). Ang II is pivotal in vascular

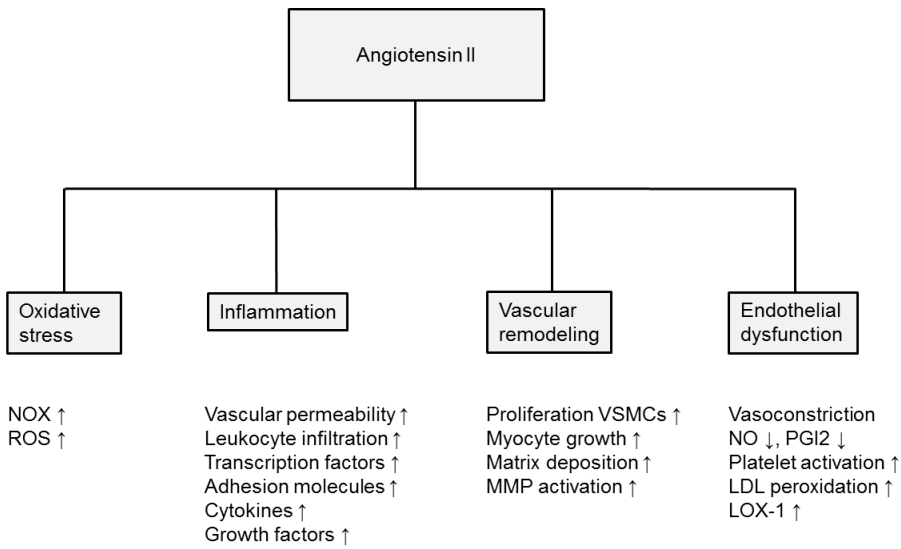

Figure 6. Angiotensin II and its effects in the development of atherosclerosis. Nox, NADPH oxidase; ROS, reactive oxygen species; MMP, matrix metalloproteinase; VSMCs, vascular smooth muscle cells; NO, nitric oxide; PGI2, prostaglandin I2 (also called prostacyclin); LDL, low-density lipoprotein and LOX, lectin-like oxidized low-density lipoprotein receptor. Figure modified from Volpe M, 2012 (4).

remodelling as it induces the expression of a number of growth factors in VSMCs (105). Ang II has been observed to induce a dose dependent increase in IL-6 in rat VSMCs (106) and to modulate vascular cell migration, decrease VSMC apoptosis (107) and alter ECM composition (108). Additionally, Ang II may increase TF expression in monocytes, ECs and VSMCs (10, 11), and conversely treatment with RAAS blockade can diminish TF in plasma and in monocytes (12). Figure 6 summarizes Ang II and its effects in the progression of atherosclerosis.

Some of the clinical effects of ACE inhibition therapy may possibly be caused by interrupting Nox-derived ROS. Studies have shown an antioxidant effect of AT1R blockers. Indeed, direct inhibition of Nox and others ROS modulators has emerged as an attractive strategy to improve ED and vascular damage in hypertensive patients (109).

1.4.5.1 Antihypertensive therapies addressing inhibition of the RAAS

Evidence supports a positive effect of RAAS inhibition (110). Some studies suggest that ACE inhibitors improve EC function, both in hypertensive patients and in patients with CHD (111, 112). Large scale studies of ACE inhibition post myocardial infarction (MI) and in heart failure demonstrated convincing evidence of reductions in the risk of recurrent MI (19, 20). Later, trials were designed to test the hypothesis that ACE inhibition in patients with CVD, but no heart failure, reduced the risk of atherosclerotic events. Indeed, the HOPE trial showed convincingly that treatment with ramipril in patients with high risk for vascular disease, without heart failure, reduced the risk of stroke, myocardial infarction and death (9). The EUROPA study demonstrated that treatment with perindopril in patients with stable CHD, reduced the risk for cardiovascular events (113). In contrast, the PEACE trial failed to confirm that patients with known CHD had a benefit of ACE inhibitors, in addition to modern conventional therapy (114). The results in that study might have been due to the low number of hard endpoint events (myocardial infarction or death). The lack of efficacy observed might simply have been affected by the enrolment of a low-risk population and by a high proportion of patients treated by statins (70%), compared to HOPE (29%) and EUROPA (56%). The QUIET study also failed to confirm a decrease in overall mortality rate after treatment with an ACE inhibitor (115). This outcome may partly have been due to enrolment of a low-risk population with both normal LDL cholesterol and body mass index. It is to be noted that, both the PEACE and QUIET trials were characterized by a very high proportion of patients previously subjected to at least one revascularization procedure (72% and 100%, respectively).

1.5 HYPERTENSION

Hypertension is globally the most common cause of morbidity and mortality. In the vascular system hypertension entails arterial remodelling and ED. Common to these processes are alterations in ECs and VSMCs to a vasoconstrictor, mitogenic, profibrotic, promigratory and proinflammatory phenotype influenced by oxidative stress.

Convincing evidence indicates that patients with hypertension have ED, both in the macrocirculation (conduit arteries) and microcirculation (arterioles, 10-150µm, and small arteries, 150-300 µm). The microvessels are known to be the main site of vascular resistance. Folkow described that already a small reduction in vessel diameter has a major effect on resistance, and that this was a result of the Poiseuille´s law (116). Blood vessels undergo structural alterations during long-term hypertension. This includes remodelling and rarefaction. Remodelling is known to be responsible for the increase in vascular resistance in hypertension (117). In small arteries and arterioles remodelling demonstrates a rearrangement of the vessel wall without growth that causes a narrowing of the lumen and an increased media-to-lumen ratio known as inward eutrophic remodelling. Rarefaction is defined as a decrease in the length or small vessel numbers in a defined volume.

Oxidative stress and ED are associated with both remodelling and rarefaction (117, 118). The endothelium may be activated by various stimuli, both chemical and physical. Hypertension in otherwise healthy subjects has been related to high levels of sICAM-1 and IL-6 (119), indicating that hypertension may contribute to an inflammatory state and to atherosclerosis. When stimulated the endothelium releases endothelium-contracting factors (EDCF) and endothelium-derived relaxing factors (EDRF), which exert effects on the underlying VSMCs. The balance between these factors determines the tone of the VSMCs. The most significant EDRF is NO, but also prostaglandin I2 (PGI2, or prostacyclin) and endothelium-derived hyperpolarizing factors are important vasodilator signals. The ECs secrete several EDCFs. One of the most important is Ang II, but also endothelin and cyclooxygenase (COX)-derived prostanoids are significant vasoconstrictors (120). In hypertension, oxidative stress induces increased production of the prostanoid TXA2 by constitutive (COX-1) and inducible (COX-2) cyclooxygenases, which leads to increased vasoconstriction and reduced endothelium-dependent vasodilation (121). It is to be noted that COX itself can produce ROS by oxidizing NADPH (121). Hence, ROS are upstream and downstream of the COX-prostanoid system.

During long-term BP elevation the ECs age prematurely and their turnover is accelerated. Eventually they are replaced by regenerated ECs (122). Importantly, the replaced endothelium has a reduced ability to release EDRFs, and in particular NO. The result of blunted NO release is a weakening of the brake to EDCFs and an endothelium-dependent contraction (120). This ED in micro- and macrocirculation also entails platelet aggregation, up-regulation of adhesion molecules, and growth of VSMCs (122, 123). These changes in the ECs contribute to thrombosis, inflammation, vascular remodelling and, in the end, atherosclerosis.

1.5.1 Shear stress and circumferential stretch

The pulsatile BP causes the blood vessels to be subject to a constant shear stress and circumferential stretch. The frictional force during the flow of blood in the vessels causes wall shear stress while the pulsation generates circumferential stretch, perpendicular to the blood flow. Under physiologic conditions, pulsatile forces with a clear direction (laminar

shear stress and circumferential stretch) cause only a transient signalling of proinflammatory and proliferative pathways, and these pathways become down-regulated when such directed forces are maintained. On the other hand, pathologic conditions with forces that are either too high or too low or without a direction (such as disturbed flow and undirected stretch at branch points) induce sustained signalling of proinflammatory and proliferative pathways (124). How the vessel wall responds to these forces is of vital importance, and a number of integrins, receptors and ECM components have been extensively studied (125).

1.5.1.1 Shear stress

In linear parts of the vessels the blood flow is laminar and shear stress is directed and high. In contrast, in curvatures and branches the blood flow is irregular and disturbed, which results in low shear stress. Persistent laminar blood flow and high shear stress protects against atherosclerosis by up-regulating the expressions of protective EC genes and proteins. In contrast, disturbed flow with low shear stress activates EC genes and proteins promoting atherosclerosis.

The most important flow mediated vasodilator is NO, which is generated from endothelial NO synthase; but also PGI2 is an important vasodilator (126). During steady laminar shear stress NO and PGI2 are up-regulated, inducing vasodilatation and inhibition of platelet aggregation, and prothrombotic molecules such as TF are down-regulated (127). The main inducer of fibrinolysis, t-PA, may be shear stress regulated, and t-PA secretion by ECs increases with increasing shear stress (128). Also, thrombomodulin (TM) is up-regulated by high shear stress (129). Laminar steady high shear stress has been shown to down-regulate AT1R in an NO dependent manner (130). In addition, AT1R antagonists reduce oxidative stress in human hypertension (131).

Low or reversing shear stress predisposes to atherosclerosis. The low flow or flow reversal causes the production of superoxides by Nox, which scavenge NO, leading to a decrease in NO bioavailability, generation of peroxynitrite and ED. (132). Peroxynitrite reduces vasodilation by reducing NO and also by decreasing the bioavailability of PGI2 (by nitration of PGI2 synthase) (133). Peroxynitrite also causes endothelial NO synthase uncoupling (by oxidizing tetrahydrobiopterin, the cofactor of endothelial NO synthase) (134). Consequently, low shear stress induces a proinflammatory and prothrombotic state through several mechanisms.

1.5.1.2 Circumferential stretch

Elevated BP causes a pressure that is perpendicular to the flow direction, and long-term hypertension induces vascular remodelling. This will lead to increased wall thickness and an augmented resistance in small vessels. The cardiac pulsative flow generates stretch on both ECs and VSMCs.

An important effect of the pulsatile character of BP is the release of Ang II and a subsequent increase of oxidative stress in ECs (135). Studies have also suggested that circumferential

stress activates AT1R, without involvement of An II (136). In VSMCs, the mechanical stress by circumferential stretch activates integrins through interaction with ECM and results in actin polymerization (137).

1.5.2 Microvascular (capillary) rarefaction

During chronic hypertension, the endothelium loses its function, and the microvessels will be constricted, unperfused and eventually the vessels disappear. This phenomenon is known as microvascular rarefaction (138). Rarefaction exists in two subforms (139):

- Functional rarefaction: Refers to a reduction in the actual number of vessels perfused.
- Structural rarefaction: Refers to a decrease in the actual number of vessels .

ED has been shown to be associated with vessel rarefaction (140). During hypertension, peripheral resistance is increased by microvascular rarefaction and a significant component of the increase in total peripheral resistance may be due to vessel rarefaction (138).

Imbalanced angiogenesis (i.e. impaired formation of microvessels), contributes to rarefaction, and ageing has been shown to be associated with impaired angiogenesis (141). NO has been shown to be implicated in angiogenesis, and microvascular rarefaction in hypertension may partially be due to reduced angiogenesis because of impaired NO biosynthesis (142). In healthy conditions, hypoxia serves as the main trigger for angiogenesis, and chronic hypoxia may induce angiogenesis by vascular endothelial growth factor pathways (143). However, in CVD with associated decreased NO synthesis, ischemia-induced angiogenesis is generally impaired (144).

1.5.2.1 Antihypertensive therapies addressing rarefaction

Few studies have focused on how antihypertensive drugs affect the microvasculature. Studies have shown that long-term treatment with various antihypertensive drugs normalize vascular structure and reverse rarefaction (145). One study compared the effect of treatment with an ACE inhibitor and a calcium channel blocker (CCB) on the retinal microvasculature. Treatment was associated with improvement in vessel narrowing and rarefaction, and no differences were observed between the treatment regimens (146). In another study, an ACE inhibitor restored the structure of arterioles and small arteries in hypertensive subjects, whereas a β-blocker did not (147). The ability to normalize the structure in the microvessels might be restricted to drugs with vasodilator capacity, such as ACE inhibitors and angiotensin receptor blockers (ARB)s, excluding drugs reducing cardiac output, such as β-blockers and diuretics (145). In support for this assumption are two studies with increased vascular area and capillary density during treatment with prazosin, an alpha 1-adrenoceptor blocker (148, 149), but the results are inconclusive, since doxazosin, another alpha 1-adrenoceptor blocker inhibited EC adhesion, migration and invasion (150). The drug class ARB also has vasodilator capacity and can increase microvessel density. In a LIFE substudy, the ARB losartan reduced vascular rarefaction and hypertrophy, compared to a β-blocker (151).

The proangiogenic contribution of ACE inhibitors on rarefaction has been examined in animal models, and drugs targeting the RAAS induce angiogenesis in most animal studies. These effects are probably mediated by bradykinin, and the generation of pathways including NO and vascular endothelial growth factor (139).

1.5.3 Hypertension and endothelial dysfunction and the RAAS

Derangements of the RAAS contribute to elevated BP and target-organ damage. Chronic inflammation in the vessel is considered to be the link between hypertension and atherosclerosis, but evidence of a causal link between oxidative stress and hypertension is not convincing, and definitive proof is still lacking.

Activation of the RAAS is a major cause of hypertension. Studies in essential hypertension have demonstrated that systolic and diastolic BP relate positively with biomarkers of oxidative stress and negatively with antioxidant levels (152). Vascular Nox activity is increased in hypertension and is highly sensitive to Ang II and aldosterone. In small arteries of hypertensive patients, the ECs and VSMCs expression of Nox 1, 2, 4 and 5 generate increased production of ROS (153). Measurements of ROS production in VSMCs resistance arteries of hypertensive patients have shown increased levels of superoxide anion and hydrogen peroxide and up-regulated Ang II-stimulated redox signalling, when compared to conditions in normotensive subjects (154).

1.5.3.1 Antihypertensive therapies addressing endothelial dysfunction

Interpretation of the effects on ED of ACE inhibitors and other antihypertensive drugs are confounded by the simultaneous reduction in BP as decreased BP per se may have the ability to reduce atherosclerotic events.

Intravenous administration of the ACE inhibitor perindopril restored normal coronary artery vascular response to endothelial stimuli in hypertensive patients (111). In agreement, the TREND study showed that the ACE inhibitor quinapril decreased vasoconstriction to acetylcholine in coronary arteries in subjects with known CHD (112). Perindopril increased FMD in hypertensive patients, while the other antihypertensive agents did not (CCBs, first and third generation β-blocker and ARB). Perindopril but also CCBs and ARB reduced oxidative stress and increased plasma antioxidant capacity (155). These findings are in accordance with the BANFF study. This study demonstrated that quinapril improved FMD in patients with CHD. On the other hand, no change was seen with the other antihypertensive drugs (156). In two studies comparing ARB to CCBs, the reduction in BP was equivalent, while the ARB reduced atherosclerosis, and the CCBs did not (157, 158). In yet another study comparing an ARB with a CCB in hypertensive patients, the group receiving ARB demonstrated improved endothelial function and an associated reduction in oxidative stress. The CCB did not have this effect (159). One study examined the effects of inhibiting the RAAS by comparing ACE inhibition to ARB in hypertensive patients with no effects on markers for inflammation, coagulation, or endothelial function (160).

1.6 HYPERLIPIDAEMIA

Hyperlipidaemia is known as an excess of lipoproteins in the blood, consisting of cholesterol, cholesterol esters, apolipoproteins, phospholipids, and triglycerides. Based on density, they are divided into five major classes, (Table 1).

Table 1. The five lipoprotein classes, sorted by increasing density.

Class of lipoprotein	CM	VLDL	IDL	LDL	HDL
Density (g/mL)	< 0.940	0.940-1.006	1.006-1.019	1.019-1.063	1.063-1.210
Major apolipoproteins	B48, C, E	B100, C, E	B100, E	B100	A, C, E
Diameter (nm)	80-1200	30-80	25-50	18-28	5-15
Free chol-esterol (wt %)	1-3	4-8	4-8	6-8	3-5
Cholesterol esters (wt %)	2-4	16-22	20-26	45-50	15-20
Phospholipid (wt %)	3-6	15-20	20-24	18-24	26-32
Triacylglycerol (wt %)	80-95	45-65	26-36	4-8	2-7

CM, chylomicrons; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein and wt, weight.

Primary hyperlipidaemias are genetic disorders, while secondary hyperlipidaemias arise due to endogenous or exogenous causes. Endogenous causes can be disorders like diabetes mellitus, thyroid disease, kidney disease, liver disorders or Cushing's disease. Exogenous causes are obesity, lack of exercise, unfavourable diet or excessive alcohol consumption.

Primary (or familial) hyperlipidaemias are classified into five different types according to the Fredrickson classification (22), which was later adopted by the WHO (Table 2).

1.6.1 Familial combined hyperlipidaemia

FCHL is a common inherited cause of hypercholesterolemia caused by a number of gene polymorphisms (161). The prevalence of FCHL is estimated to be 0.5-2.0%, and the prevalence of CHD in patients less than 60 years old is estimated to be as high as 20% (162). FCHL is characterised by increased levels of apolipoprotein B100 containing lipoproteins:

Table 2. Primary (or familial) hyperlipidaemias according to the Fredrickson classification.

Phenotype	Lipoproteins elevated	Cholesterol concentration	Triglyceride concentration	Frequency (%)
I	Chylomicrons	Normal to ↑	↑↑↑↑	<1
IIa	LDL	↑↑	Normal	10
IIb	LDL and VLDL	↑↑	↑↑	40
III	IDL	↑↑	↑↑↑	<1
IV	VLDL	Normal to ↑	↑↑	45
V	VLDL and chylomicrons	↑ to ↑↑	↑↑↑↑	5

VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein and HDL, high-density lipoprotein.

VLDLs, intermediate-density lipoproteins (IDLs) and LDLs. FCHL is frequently associated with reduced HDL cholesterol, and insulin resistance (163, 164). Also, patients with FCHL have a predominance of small dense LDL cholesterol, which is a diagnostic indicator for CHD risk (165). VLDL activates NF-κB in vivo and in vitro, and this is associated with an augmented expression of NF-κB-regulated molecules like ICAM-1, VCAM-1 and TNF-α (25). VLDL and remnant particles may induce a procoagulant phenotype mainly by increasing platelet activation (166). FCHL is characterized by impaired endothelium reactivity and hypercoagulability (23, 167), increased PAI-1 levels and a chronic inflammation (25-27). Thus, patients with FCHL exhibit ED, disturbed coagulation, impaired fibrinolysis and on-going chronic inflammation. These characteristics can all be responsible for the increased incidence in cardiovascular events in FCHL subjects.

1.6.2 Familial hypercholesterolemia

FH is a disorder that is caused mainly by genetic disturbance in the receptor for LDL, and inherited by an autosomal dominant pattern. The LDL receptor (LDLR) function is disturbed and the levels of LDL are dramatically increased (168). The heterozygous condition affects at least 1 in 500 individuals (0.2%). Patients with FH are affected by increased CHD and premature death (168). If not treated, about 50% of men and 20% of women are expected to develop CHD before the age of 50 years (168).

FH is associated with impaired endothelial reactivity (28), and hyperactive platelets in humans (169). Hypercholesterolemia has been shown to exhibit a prothrombotic state without any influence on fibrinolysis (29, 30). The cause of the procoagulant state is unknown, but one mechanism might be that elevated levels of oxLDL in FH have been shown to cause an increase the expression of active TF in human monocytes (11, 170). In one of these studies the authors found that TF inhibition reduced the levels of IL-6, indicating crosstalk between inflammation and coagulation during FH. They proposed that oxLDL may lead to induced expression of both TF and IL-6, and the development of a prothrombotic and proinflammatory state. Consistent with these studies, patients with high LDL concentrations exhibited an increased TF plasma activity (171). Accumulating evidence demonstrates that hypercholesterolemia activates the RAAS, and one possible mechanism might be the observation that oxLDL up-regulates ACE and AT1Rs (172). Also, studies have shown that hypercholesterolemia is associated with hypertension (173). FH is not associated with insulin resistance (174).

1.6.3 Hyperlipidaemia and the RAAS

The first sign of atherosclerosis is the fatty streaks that appear during the first or second decade of life. The exact mechanisms are unclear, but according to *the oxidation hypothesis* of atherosclerosis, LDL transmigrates from the lumen into the vessel wall. The LDL particle is retained when it undergoes oxidative modification by ROS or enzymes (175). OxLDL has itself proinflammatory effects and stimulates proinflammatory signalling pathways (176). OxLDL may also activate ECs and stimulate the up-regulation of vWF and adhesion molecules. The presence of chemokines like MCP-1 attracts leukocytes into the intima that promotes an activation of the immune system. Leukocytes are continuously recruited into the subendothelial area, and monocytes then differentiate into macrophages. In the vessel wall, macrophages take up oxLDL via the scavenger receptors, a process that eventually leads to the transformation of macrophages into foam cells (177).

In VSMCs, oxLDL interacts with Toll-like receptors (TLR) 4 and CD36, which enhances the production of cytokines, chemokines and growth factors, resulting in recruitment monocytes and differentiation of macrophages (178).

Lectin-like oxidized LDLR (Lox-1) is a receptor on inflamed ECs and VSMCs, promoting the uptake of oxLDL. The activation of Lox-1 is stimulated ROS, oxLDL and Ang II (179). Lox-1 also causes an up-regulation of the AT1R (180), demonstrating a potential cross-talk between Lox-1 and AT1R.

Both LDL and oxLDL can activate the RAAS and their receptors in human ECs (172), and hypercholesterolemia and elevated Ang II levels in hypertension can accelerate the development of ED and atherosclerosis (181). These data indicate a link between hypercholesterolemia, hypertension and atherosclerosis.

Other lipoproteins, such as the triglyceride-rich lipoprotein VLDL and IDL also have considerable atherogenic potential (166). These lipoproteins may undergo oxidative

modification like that of LDL, and some evidence suggests that VLDL particles may have inflammatory effects in vascular cells (25).

1.7 INSULIN RESISTANCE

Insulin resistance is a disorder in which the body fails to respond to insulin and exhibits dysregulated insulin stimulated uptake of glucose in skeletal muscle and adipocytes, leading to high levels of insulin and blood glucose. Insulin resistance is a risk for type 2 diabetes. The prevalence of insulin resistance is increasing globally, in particular in hypertensive and obese subjects.

Insulin is secreted from pancreatic β-cells as a result of glucose stimulus. When insulin binds to the insulin receptor a series of intracellular events takes place, referred to as the insulin signalling cascade. It all culminates in glucose transporter type-4 (GLUT-4) translocation, from the cytoplasm to the cell membrane (figure 7). GLUT-4 translocation involves downstream signalling via the insulin receptor and insulin receptor substrate-1 pathway, but also through phosphatidylinositol 3-kinase and ak strain transforming (AKT, also referred to as protein kinase B) pathway (182).

Most insulin resistance states are a consequence of defects in the upstream signalling pathway. Insulin resistance seldom exists isolated; instead it often coexists with other cardiovascular risk factors, the metabolic syndrome in particular(183).

1.7.1 Insulin resistance and the RAAS

Clinical trials implicate that the RAAS has a potential role in insulin resistance as well as in type 2 diabetes, while blockade of the RAAS has been shown to prevent both states (9, 184, 185). A meta-analysis has shown that ARB and ACE inhibitors decrease the incidence of new onset diabetes by 23% and 27%, respectively (186). Ang II stimulation of AT1R results in vasoconstriction and a decrease in microvascular blood flow, and these hemodynamic changes attenuate the delivery of both glucose and insulin to skeletal muscle. Data also indicates that Ang II mediates its effects by direct, non-hemodynamic actions. The activation of AT1R by Ang II induces ROS by Nox. ROS on its own, or by activating MAP kinase or NF-κB signalling, then inhibits the insulin signalling pathway. The result is inhibition of the translocation of GLUT-4, which remains in the cytoplasm (figure 7).

1.7.1.1 The ACE2-Ang-(1-7)-Mas axis

Studies have shown that Ang-(1–7), which mediates its effect by interacting with the Mas receptor, has a positive regulatory impact on insulin signalling via stimulation of AKT (187). Hence, the balance between the two axes ACE-Ang II-AT1R and ACE2-Ang-(1-7)-Mas is a crucial factor in insulin resistance, and a ratio of the peptides Ang II and Ang-(1-7) is likely to be a determinant of the overall activity.

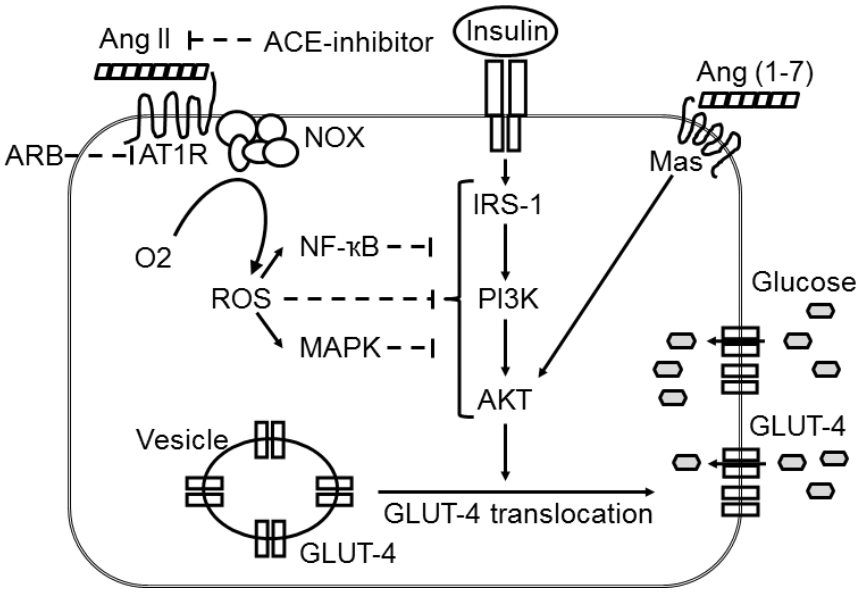

Figure 7. Ang II inhibits the insulin signalling pathway. RAAS induce hemodynamic changes, i.e. vasoconstriction and reduced blood flow in microvessels, but also non-hemodynamic action, while causing the formation of ROS, oxidative stress and subsequently insulin resistance. The axis of ACE-Ang II-AT1R augments an increase in ROS and inhibits the insulin signalling pathway, causing inhibited translocation of GLUT-4. Conversely, the axis of ACE2-Ang-(1–7)-Mas shows a positive regulatory effect on insulin signalling via stimulation of AKT. Hence, the balance between these two arms is a crucial factor in insulin resistance. Ang, angiotensin; RAAS, renin-angiotensin-aldosterone system; PI3K, phosphatidylinositol 3-kinase; AKT, ak strain transforming or protein kinase B); ROS, reactive oxygen species; NF-*κ*B, nuclear factor kappa B; Nox, NADPH oxidase; MAPK, mitogen-activated protein kinase; AT1R, angiotensin type 1 receptor; Ang-(1-7), angiotensin-(1-7); IRS, insulin receptor substrate; ARB, angiotensin receptor blocker; ACE, angiotensin converting enzyme and GLUT, glucose transporter. Figure modified from Henriksen EJ, 2013 (188).

1.7.1.2 Aldosterone

Aldosterone interferes directly with insulin signalling. Indeed, studies have shown that aldosterone may predict the development of insulin resistance. In the vasculature, MRs have been described on both ECs and VSMCs (73). Activation of the AT1R is the primary stimulator of adrenocortical release of aldosterone, and inhibition of RAAS by ACE inhibitors or ARBs decrease plasma aldosterone levels, while dietary salt restriction increases aldosterone production and vascular insulin resistance (189). Excess aldosterone and consequently MR activation reduces NO production and increases ROS, leading to ED, VSMC hypertrophy, fibrosis and inflammation (190).

Aldosterone has inhibitory effects on insulin signalling by inducing insulin resistance in VSMCs through insulin receptor substrate-1 expression and AKT signalling (191). In ECs,

aldosterone and MR activation increase expression of ICAM-1 and Nox-4, leading to oxidative stress and decreased NO bioavailability (73). Hypertension and obesity are associated with insulin resistance. Aldosterone has also been shown to reduce insulin secretion directly in pancreatic cells (192).

1.7.1.3 Renin, prorenin and renin-prorenin receptor

Blockade of renin improves insulin sensitivity. In subjects with the metabolic syndrome treatment with a renin inhibitor improved insulin sensitivity, as compared to an ARB (193). In a rat model of RAAS activation, a renin inhibitor led to improved insulin resistance and signalling, and also improved glucose uptake in skeletal muscle (93). Improvements in insulin signalling may also be due to decreased levels of Ang II and aldosterone, and this makes it hard to determine whether the effects of renin inhibition are through effects of renin blockade, or through the effects of decreasing downstream levels of Ang II or aldosterone.

1.8 HAEMOSTASIS

Haemostasis consists of the platelet system, the coagulation and the fibrinolytic system, and is often classified as:

- Primary haemostasis:
  - Vascular contraction.
  - Platelet activation and aggregation.
- Secondary haemostasis:
  - Coagulation. Consists of TF (extrinsic) and contact (intrinsic) pathways.
  - Fibrin formation and clot stabilitation.
- Fibrinolysis:
  - Removing of the clot, once blood vessel integrity has been restored.

Haemostasis is a fine-tuned balanced system, and in normal conditions the factors for haemostasis are present in inactivated forms in circulating blood and can typically be activated upon vessel inflammation or damage. During inflammatory states involving ED disturbances at several levels of the haemostatic system, this may result in pathophysiological changes in primary and secondary haemostasis or fibrinolysis.

1.8.1 Coagulation

The TF (extrinsic) and contact (intrinsic) pathways divide the coagulation cascade into two parts.

1.8.1.1 Tissue factor pathway (extrinsic)

The TF (or extrinsic) pathway, the major inducer of the coagulation cascade, is initiated when TF is bound to FVII-FVIIa (see figure 8). The TF-FVIIa complex cleaves FX into FXa, and FIX into FIXa, respectively. FXa then forms the prothrombinase complex when associated with FVa and Ca^{2+} on activated platelets, resulting in thrombin formation. The reaction is

much faster (300,000-fold than baseline) in the presence of negatively charged surface phospholipids (i.e. phoshatidylserine) on activated platelet membranes. Microparticles (MPs) may also accelerate thrombin generation due to a procoagulant phosphatidylserine-rich surface that supports thrombin generation (194).

TF has for long been known to be present in the extravascular space (in subendothelial tissue) and not exposed to flowing blood, except during plaque rupture. TF is also present in circulating blood, mainly in three pools. The most important source of TF is monocytes, expressed as membrane-anchored and membrane-bound. There is no evidence that granulocytes express TF, rather they seem to acquire TF from monocytes (195). Controversial is the expression of TF in platelets. Platelets might store TF in α-granules or acquire TF from TF-containing MPs (196, 197). The second source of circulating TF is different cell-derived MPs, which are considered to be key players in atherothrombosis. The third pool, probably of minor importance, is a spliced form of TF, which is soluble and circulates in the blood (198).

The importance of the intravascular blood-borne TF is controversial. Blood-borne TF means that the coagulation cascade may be activated without contact between the blood and the extravascular space (i.e. plaque rupture). This appears to be an important contribution in intravascular thrombosis (199).

Blood-borne TF implicates that TF apparent on the surface of cells has a low activity, and under normal conditions TF is obviously in an inactive, cryptic, state. To be functionally active, and to exhibit a procoagulant activity, TF has to undergo an activation step, decryption. The molecular differences between these states are controversial and remain to be clarified. Several mechanisms have been suggested to explain the decryption step (200).

Many cytokines are responsible for expression of TF on mononuclear cells in vitro. Studies have reported that the TF expression is mostly dependent of IL-6. Inhibition of IL-6 has been shown to completely block thrombin generation, whereas other cytokines had no effect (201). IL-6 has been shown to be relevant for activation of coagulation (202). Also CRP has been shown to induce TF expression in both ECs and VSMCs (203). TF also possesses a function as a signalling receptor. TF binding of FVIIa has been shown to trigger VSMC proliferation (204).

1.8.1.2 Thrombin generation

Thrombin is generated in three phases: the initiation phase, the amplification phase and the final propagation phase. During the first phase, only small amounts of thrombin are generated and during the next two phases, and in particular in the final phase, large amounts of thrombin are formed (205):

- Initiation phase: Requires activated TF on ECs, monocytes, MPs or subendothelial TF after vascular injury. TF activates FVII and then TF-FVIIa activates FX and FIX. FXa activates FV. FXa complexes with FVa and if the stimulus is strong, enough thrombin

is formed to initiate the coagulation process. The amount of thrombin generated is under the influence of tissue factor pathway inhibitor (TFPI) activity as it rapidly inhibits FXa.

- Amplification phase: This response takes place on phospholipid surfaces, mostly on activated platelets. The stimulus is amplified as platelets are activated and accumulate cofactors on their surfaces. Activated platelets release FV, which is activated by thrombin or FXa into FVa. Circulating vWF-FVIII complexes bind to activated platelets and FVIII is released and activated into FVIIIa by thrombin.

- Propagation phase: Finally, the active proteases combine with their cofactors on phospholipid surfaces of activated platelets, but can also take place on MPs, activated ECs or VLDLs**.** The tenase complex, consisting of FIXa-FVIIIa and Ca2+ activates FX into FXa. Thereafter the prothrombinase complex, consisting of FXa-FVa and Ca2+, induces a thrombin burst that converts fibrinogen into fibrin. Thrombin activation of FXIII cross-links the fibrin monomers and stabilizes the platelet plug. Thrombin also stimulates on-going coagulation by feedback activation of FXIa.

1.8.1.3 Contact pathway (intrinsic)

Thrombin may also be activated by the contact (or intrinsic) pathway (see figure 8). The contact activation pathway is less prominent for haemostasis under healthy conditions than the TF pathway. However, states like hyperlipidaemia may lead to thrombosis via the contact pathway (206).

The contact pathway consists of FVIII to FXII. Also required are prekallikrein and the cofactor high-molecular-weight kininogen, and calcium ions. The contact pathway requires a surface with negatively charged phospholipids, often secreted from platelets. Interaction with phospholipids may also occur on certain lipoproteins, such as chylomicrons, VLDLs or oxLDLs. Thus hyperlipidaemia can promote a prothrombotic state via the contact pathway.

The contact phase is initiated as prekallikrein is converted to kallikrein, cleaving FXII to FXIIa. In turn, FXIIa then cleaves FXI to FXIa. FXIIa will also convert prekallikrein to kallikrein, through mutual activation. Kallikrein can acts upon kininogens that leads to the release of the vasodilator bradykinin. FXIa activates FIX to FIXa, in the presence of Ca^{2+}. FIXa then cleaves FX to active FXa. Activation of FXa needs the tenase complex on platelets or certain lipoproteins. FVIII is mainly produced by the liver, and circulates in an inactive state in blood in complex with vWF. The activation of FVIII to FVIIIa occurs with minute quantities of thrombin or FXa. During activation FVIII dissociates from vWF and becomes fully activated and then participates in the FX activating complex. But when thrombin concentration increases, FVIIIa is inactivated by thrombin. Hence, thrombin has a dual action upon FVIII, and regulates the extent of the amount of the tenase complex formed and consequently has an important impact on the activity of the coagulation cascade (207, 208).

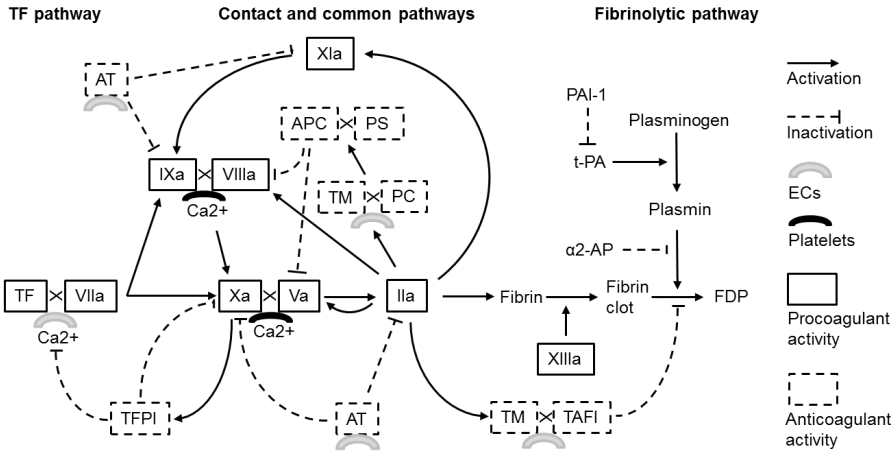

Figure 8. Blood coagulation cascade. To clarify the figure zymogens are not depicted. F, coagulation factor; Xa-Va, FXa-FVa complex or the prothrombinase complex; IXa-VIIIa, FIXa-FVIIIa complex or the tenase complex; IIa, FIIa or thrombin; XIIIa, FXIIIa; PC, protein C; APC, activated protein C; PS, protein S; TF-VIIa, tissue factor-FVIIa complex; AT, antithrombin; TM, thrombomodulin; t-PA, tissue plasminogen activator; TFPI, tissue factor pathway inhibitor; Xia, FXIa; TAFI, thrombin activatable fibrinolysis inhibitor; α2-AP, alpha 2-antiplasmin; PAI, plasminogen activator inhibitor; FDP, fibrin degradation products and Ca2+, calcium ion. Figure modified from Bouma BN, 2006 (209).

1.8.1.4 The role of thrombin

Thrombin is of vital importance in the coagulation cascade. The main action is to act as a procoagulant by converting fibrinogen to fibrin monomers. Thrombin also exercises an anticoagulant role by binding to TM at the intact endothelium and to promote activation of the protein C (PC) pathway. APC then inactivates FVa and FVIIIa, two essential cofactors for FXa and FIXa, thereby down-regulating thrombin generation (210). Thrombin also has a central role in inflammatory response and stimulates different types of cells in the vasculature and in blood, including ECs, VSMCs and platelets. Effects of thrombin are mediated by their counter-receptors termed protease-activated receptors (PAR)s. PARs are G protein-coupled receptors, and four receptors have been identified (PAR 1-4), and PAR-1 has been identified as the main thrombin receptor in vessels and on platelets.

Thrombin signalling in platelets contributes significantly to haemostasis and thrombosis. It is the most effective platelet activator and causes the platelets to change shape and secrete adenosine diphosphate, serotonin, TXA2, chemokines, growth factors, mobilization of P-selectin and CD40L, activation of αIIbβ3 and, ultimately, platelet aggregation (211). Platelets in humans express PAR-1 and PAR-4. PAR-1 seems to be the main platelet receptor for thrombin, and can activate platelets even at very low thrombin concentrations. Thrombin also promotes the release of EC MPs that seem to be critical in vascular pathophysiology (212). Once trace amounts of thrombin have been generated this is then able to activate FV, FVIII, FXI and FXIII.

In the healthy endothelium, thrombin activates PAR-1 and stimulates the production of prostacyclin and NO, leading to vasodilatation and activation of fibrinolysis by the release of t-PA (213). During inflammation and dysfunction of the endothelium PAR expression is increased in the endothelium, priming the response of ECs to thrombin and shifting ECs to a proinflammatory phenotype, inducing synthesis and release of PAI-1, contributing to impaired fibrinolysis (214). In pathophysiological conditions PAR-1 activation causes morphological changes, an increased vascular leakage and the release of proinflammatory cytokines, as well as up-regulation of adhesion molecules (215). In particular the synthesis of IL-6 seems to mediate the transition of the inflammatory process in the vessels from an acute to a chronic phase (216). Activation of PAR-1 also mobilizes p-selectin and vWF from Weibel-Palade bodies, promoting EC rolling and subsequently firm adhesion of both platelets and leukocytes.

In healthy arteries PARs are preferably expressed in ECs, while their expression in VSMCs is limited. In hypertension and atherosclerotic vessels PAR-1 is up-regulated in VSMCs (214). This implies that PARs on VSMCs have a more prominent role under pathological conditions. In states associated with ED, PARs in VSMCs mediate contraction, proliferation, migration, hypertrophy and the production of ECM (214).

Thrombin activatable fibrinolysis inhibitor (TAFI) originates from the liver, and is transported in the plasma as a zymogen, bound to plasminogen. The thrombin-TM complex activates TAFI preventing up-regulation of plasminogen binding and activation and reduces the binding of plasminogen, plasmin and t-PA to fibrin and thus slowing clot lysis (217).

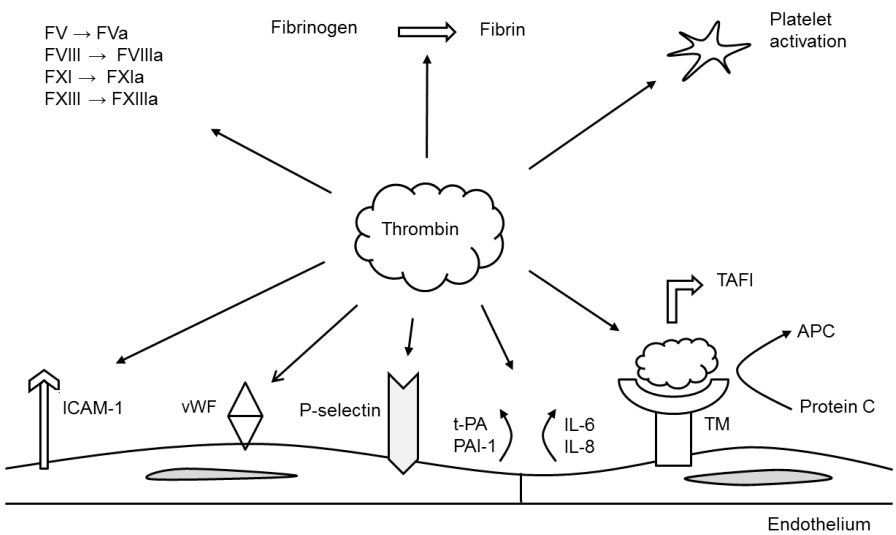

Figure 9. Role of thrombin in the coagulation system. ICAM, intracellular cell adhesion molecule; PAI, plasminogen activator inhibitor; vWF, von Willebrand factor; IL, interleukin; TAFI, thrombin fibrinolysis activatable inhibitor; TM, thrombomodulin; APC, activated protein C and F, coagulation factor. Figure modified from Davidson SJ, 2013 (218).

Altogether, in healthy conditions thrombin and PAR-1 activation causes endothelium dependent vasodilatation. On the other hand, in pathological conditions, thrombin regulates several components, which induces a procoagulatory and proinflammatory state (figure 9).

1.8.2 Fibrinolysis

The fibrinolytic system aims to dissolve and remove clots within the circulation. The zymogen plasminogen is released from the liver and is accumulated in fibrin-rich clots. Upon activation plasminogen is cleaved to plasmin by a variety of enzymes. The main regulator of fibrinolysis is t-PA released from ECs. In endotoxemia and cytokinemia the fibrinolytic system is transiently activated but thereafter depressed due to elevated PAI-1 levels. PAI-1 appears in human blood in 3 different forms, active, latent (representing an inactive form) and complexed to t-PA or urokinase-PA. Concentration of PAI-1 exceeds t-PA by a 4:1 ratio and PAI-1 binding to t-PA or urokinase-PA occurs in a ratio of 1:1, thereby effectively limiting fibrinolysis. When PAI-1 levels are increased the t-PA half-life is considerably shortened, and a negative correlation is present between PAI-1 and plasmin-antiplasmin (PAP) complexes. The complex of t-PA/PAI-1 is stable, and eliminated from the circulation by the liver. PAI-1 half-life is around 2-4 h, and has a diurnal variation with a peak at 3 a.m. and falls during morning to a lowest level in the afternoon (219). TNF-α is important in the induction of the fibrinolytic responses, while IL-6 have been shown to be most relevant for coagulation activation (202), but also enzymes like kallikrein, FXIa and FXIIa may convert plasminogen into plasmin. Plasmin acts by degrading fibrin into d-dimers. The main inhibitor of fibrinolysis and t-PA is PAI-1, while α2-antiplasmin and α2-macroglobulin inhibits plasmin.

1.8.3 Fibrinolysis and the RAAS

The RAAS may influence the fibrinolytic system since Ang II have been reported to stimulate the production of PAI-1 by ECs and VSMCs, and bradykinin (which is degraded by the ACE) to stimulate the production of t-PA (220). Ang II can increase protein activity of TF and PAI-1 activities and the activities were increased already after 4 h (10). Ang II has also been observed to increase PAI-1 and t-PA activator messenger RNA in rat ECs and aortic VSMCs (221). Additional experiments have demonstrated that Ang IV caused an increased PAI-1 expression and the response exhibited a fast time and a dose dependence, while the induction of PAI-I messenger RNA expression produced by Ang II was evident within 1 h and is maximal at 4 h (222). Blocking the RAAS by ACE inhibition after myocardial infarction has been shown to improve the fibrinolytic balance (223).

Ang II infusion also increases the expression of messenger RNA PAI-1 in the rat (224). In normotensive subjects and in hypertensive patients, Ang II infusion resulted in an increase in PAI-1 antigen, whereas no changes occurred regarding t-PA antigen (225). In conflict, Ang II did not involve any changes in PAI-1 antigen or activity in healthy subjects in other studies (226, 227). Thus, a link between PAI-1 and Ang II is at present unclear.

1.9 CROSSTALK BETWEEN INFLAMMATION AND COAGULATION

Evidence suggests that inflammation and haemostasis are tightly interrelated and that these two systems affect each other in a bidirectional way, i.e. inflammation may lead to activation of the haemostatic system, while the haemostatic system has an impact on the inflammatory activity (228).

Cross-talk between inflammation and haemostasis helps to explain prothrombotic states in inflammatory environments, such as the marked inflammatory response following cardiac surgery, when an uncontrolled inflammatory response leads to a profound disturbance in the coagulation system. In contrast, activated coagulation factors can directly stimulate an inflammatory response. In particular thrombin can activate receptors on mononuclear cells or ECs, which may induce cytokines and cause an inflammatory response.

1.9.1 Inflammation induced coagulation activation

Cytokines are responsible for inflammatory induced activation of haemostasis. A number of trials show the vital importance of the cytokine IL-6 in the activation of coagulation, and also important roles of IL-1, IL-8, TNF-α, and MCP-1 (201, 229). The inflammatory mediators trigger disturbance of the haemostatic system through different mechanisms:

- Endothelial dysfunction
- Activation of platelets
- Activation of coagulation cascade through TF
- Suppressed anticoagulant pathways
- Impaired fibrinolytic activity

1.9.1.1 Endothelial dysfunction

Intact endothelium offers an anti-thrombotic surface, thereby preventing coagulation activation. Under healthy conditions ECs produce components with anti-inflammatory, anticoagulant and profibrinolytic properties, but in inflammatory conditions, ECs release procoagulant and anti-fibrinolytic mediators (like vWF, TXA2 and PAI-1). Activated ECs express TF and adhesion molecules, which are of vital importance in mediating the interaction of leukocytes and platelets with the ECs, and thus in activating the coagulant system and in promoting an inflammatory response. The cytokines IL-1, IL-6, IL-8, TNF-α and the chemokine MCP-1 have a key role in mediating the procoagulant changes in ED (201, 229).

1.9.1.2 Activation of platelets

In healthy conditions platelets circulate in a resting state. Intact ECs release NO and PGI2 to maintain disc-shape resting platelets. Several factors contribute to the activation of platelets. EC dysfunction activates platelets via the imbalance of increased production of vWF and TXA2 and the simultaneous decrease of NO and PGI2. Platelets can also be directly activated by endotoxins, cytokines such as IL-6 or PAF and VLDL (166, 230, 231). One of the

strongest platelet activator is thrombin, by binding on platelet receptors for thrombin (in particular PAR-1, but also PAR-4) (232).

When activated, platelets undergo a substantial shape change, and immediately express at their surface or secrete, from alpha or dense granules, proinflammatory and procoagulant substances (39). Several hundreds of different platelet derived mediators have been identified. Activated platelets synthesize TXA2, while adenosine diphosphate and ATP are released from dense granules. These mediators amplify activation of platelets and recruit circulating platelets. Activated platelets also express receptors for β1 and β3 integrins, mediating platelet adhesion and aggregation.

When platelets and ECs are activated, P-selectin is translocated to the cell surface where it functions as a PSGL-1 receptor, expressed on leukocytes (and in small amounts on platelets). The subsequent P-selectin and PSGL-1 interaction in turn increases the release of cytokines and chemokines from neutrophils and monocytes. The interaction also stimulates the up-regulation of adhesion molecules and TF on ECs and leukocytes (233).

IL-1β is a pivotal mediator in the cytokine cascade and also an important activator of ECs, by inducing the cytokines IL-6, IL-8 and chemokine MCP-1 (234). Platelet IL-1β also induces up-regulation of adhesion molecules like ICAM-1 and the vitronectin receptor (αvβ3), contributing to adhesion of neutrophils and monocytes to ECs (235).

CD40L is also a key player in platelet activation. CD40L and its receptor CD40 are expressed in cells including platelets, ECs, VSMCs, T lymphocytes and macrophages (236). When activated, platelets rapidly (within seconds) express CD40L, and the interaction of CD40L and CD40 on ECs up-regulate the cytokines IL-8 and IL-6, MCP-1 and adhesion molecules, as well as increasing TF expression (237).

Human platelets appear not to express TF when activated (238). On the other hand, activated platelets generate MPs that seem to express TF on their surface and contain negatively charged phospholipids, two factors of major importance for coagulation reactions (239). Figure 10 summarizes the most common proteins released upon platelet activation.

1.9.1.3 Activation of coagulation cascade through TF

The coagulation cascade in inflammatory states is mainly mediated by TF (240). Under physiological conditions TF is not expressed or is in an inactive state in circulatory cells or ECs. However, various inflammatory signals, like the cytokines IL-6, IL-1 or TNF-α, can induce TF activation in leukocytes (i.e. monocytes and macrophages) and ECs. Also CRP seems to have a role in coagulation as CRP can induce TF activation via a NF-κB pathway in both ECs and VSMCs (203). Other mediators like oxLDL (241) and oxygen free radicals (242) can enhance TF activation. This enables active TF to be exposed to blood and bind to FVII. The complex of TF and FVIIa then contributes to the conversion of FIX and FX into the active proteases FXa and FXa, thereby generating thrombin.

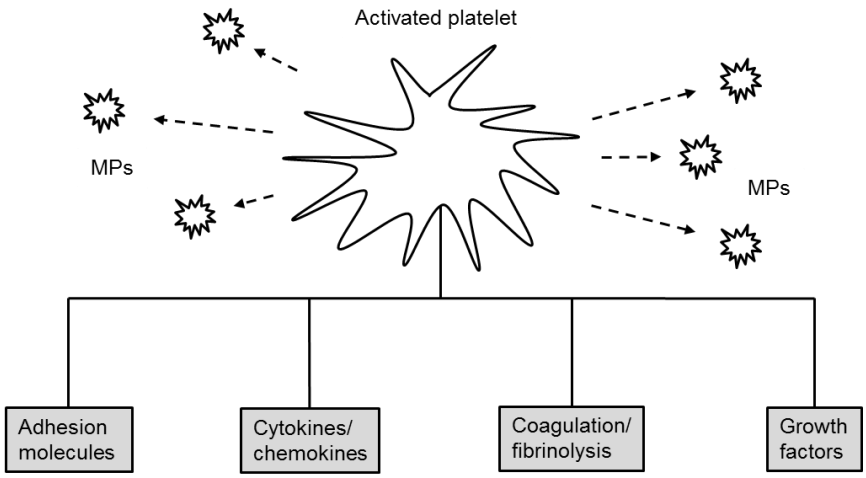

Figure 10. Activation of platelets. MPs have negatively charged phospholipids on their surfaces and expresses TF. αIIbβ3, also known as glycoprotein IIbIIIa, the fibrinogen and vWF receptor; GPIbα-IX-V, the vWF receptor; αvβ3, the vitronectin receptor; α5β1, the fibronectin receptor; α6β1, the laminin receptor; α2β1 and GPVI are the receptors for collagen; GP, glycoprotein; β-TG, beta-thromboglobulin; PAF, platelet activating factor; CD, cluster of differentiation; MCP, monocyte chemoattractant protein; IL, interleukin; PF, platelet factor; RANTES, regulated on activation, normal T-cell expressed and secreted; NAP, neutrophil-activating peptide; LT, leukotriene; vWF, von Willebrand factor; F, coagulation factor; TFPI, tissue factor pathway inhibitor; PAI, plasminogen activator inhibitor; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; VEGF, vascular endothelium growth factor; IGF, insulin growth factor; TGF-β, transforming growth factor beta and FGF, fibroblast growth factor.

Under physiological circumstances, the presence of negatively charged membrane surfaces is limited, so even if activated coagulation factors are generated, the propagation of the coagulation cascade is limited. During inflammation activated platelets and ECs express negatively charged phospholipids on their surfaces, which is a prerequisite for assembly of activated coagulation factor complexes.

1.9.1.4 Suppressed anticoagulant pathways

The mechanisms that regulate coagulation activation are antithrombin (AT), the PC system and TFPI. The purpose of these systems is to prevent clotting of blood under healthy

conditions and also to dampen the activity of the coagulation system during vascular inflammation. Figure 8 summarizes the anticoagulant pathways.

In addition to their effects on coagulation they also offer anti-inflammatory properties (243). The function of these pathways is impaired during inflammatory states and represents a mechanism in which inflammation leads to a procoagulant state (244).

1.9.1.4.1 Antithrombin

AT is produced by the liver and is the most important inhibitor of thrombin and FXa, but also FIXa, FXIa and FXIIa. Besides the anticoagulant properties AT seems to exert anti-inflammatory properties. By binding of AT to thrombin, the levels of free, active thrombin is reduced, and this contributes per se to reduced activation of platelets, leukocytes and ECs. AT also seems to have direct interaction with leukocytes by blocking interaction with ECs, i.e. blocking leukocyte adhesion and migration on ECs (245).

In inflammation, the AT pathway is impaired as a consequence of increased consumption, decreased synthesis in the liver and degradation by neutrophil elastase. Proinflammatory cytokines also cause reduced synthesis of GAGs, such as heparin sulphate, which may contribute to impaired AT function (246).

1.9.1.4.2 Protein C system

PC is a zymogen synthesized in the liver, and PC is activated when thrombin binds to TM, generating APC. This activation preferably occurs on the surface of intact endothelium. TM not only accelerates PC activation, by binding thrombin, but also directly impairs most of the procoagulant and proinflammatory effects of thrombin. The activation rate of PC is estimated to be increased 10-fold when it is adheres to the endothelial protein C receptor (EPCR) (247). APC binds reversibly to EPCR and must dissociate from the receptor before it can bind to its cofactor protein S (PS) to form the PS-APC complex that in turn inactivates FVa and FVIIIa.

The PC system is the most complex anticoagulant pathway and appears to be the most important in affecting inflammation. It is considered that the APC-EPCR complex has anti-inflammatory effects (248) by blocking the NF-κB translocation to the nucleus, resulting in decreased expression of cytokines and adhesion molecules in ECs and thereby inhibits the chemotaxis and adhesion of leukocytes to ECs. APC also stimulates fibrinolysis by forming a complex with PAI-1 and this reaction increases dramatically by vitronectin (249). TM has direct anti-inflammatory effects as TM inhibits leukocyte adhesion to the endothelium by attenuating the adhesion molecule expression via NF-κB and MAPK pathways (250). EPCR may be released as a circulating, soluble protein by IL-1, thrombin or inducible metalloproteinase in the ECs. This soluble EPCR then binds to a β2-integrin on neutrophils, and probably inhibits neutrophil trafficking (251). Additionally, the thrombin-TM complex activates TAFI, and thereby inhibits fibrinolysis. The function of the PC system is impaired during inflammatory states as both TM and EPCR have been shown to be down-regulated by inflammatory cytokines (252). Also, leukocyte activation and oxidant stress decrease the

activity of TM and neutrophil elastase may cleave TM rapidly, leading to a decreased PC activation (253) .

1.9.1.4.3 Tissue factor pathway inhibitor

TFPI can reversibly inhibit FXa, and the FXa-TFPI complex subsequently inhibits the FVIIa-TF complex. Importantly, the complex of FXa-TFPI has been shown to be a more potent inhibitor of the FVIIa-TF than TFPI itself. TFPI is secreted by ECs and attached to the endothelium via GAGs. During inflammation and exposure of proinflammatory cytokines the synthesis of GAGs is reduced and this may affect the function of TFPI. Also ROS formation inhibits TFPI binding to FXa and loss of activity (254).

1.9.1.5 Impaired fibrinolytic activity

The fibrinolytic system controls haemostasis, and the pivotal enzyme, plasmin degrades the fibrin clot. The plasminogen activators generate plasmin from plasminogen. The major inhibitor of t-PA and urokinase-PA, is PAI-1. When PAI-1 binds to the plasminogen activators, inactivation of these plasminogen activators takes place, and consequently the activity of the fibrinolysis is supressed.

The ratio between t-PA and PAI-1 modulates the fibrinolytic activity (255). The immediate response during inflammatory stimuli is a transient increase in the secretion of t-PA from Weibel-Palade bodies in ECs (256). This increase in the fibrinolytic activation is followed by a delayed suppression of the t-PA production and a sustained increase in PAI-1, resulting in a suppression of the fibrinolytic activity. The major regulators of PAI-1 activity at inflammatory sites seem to be cytokines, such as IL-6, IL-1β and TNF-α (219, 257). CRP has also been shown to stimulate the expression of PAI-1 (34). Alpha granules in platelets also contain PAI-1 that can be released upon activation, which increases PAI-1 and thereby contributes to suppression of fibrinolysis in inflammatory states. High levels of PAI-1 and t-PA have been shown to predict development of a first cardiovascular event (258).

1.9.2 Coagulation induced inflammatory activation via PARs

Inflammation induces activation of the coagulation cascade, and the coagulation pathway triggers an intracellular inflammatory pathway. This cross-talk causes a positive feedback loop which amplifies an inflammation-thrombosis circuit (259).

The coagulation factors can induce vascular inflammation by their binding to PARs (260). PARs are present in ECs, leukocytes, platelets, fibroblasts and VSMCs (260). An exceptional characteristic of PARs is that these receptors carry their own ligand, which is unmasked until the receptor is cleaved. Thrombin is known to be the most essential player in activation of PARs, and can activate PAR-1, PAR-3 as well as PAR-4. FXa transmits activation of PAR-1 to PAR-3, while TF-FVIIa transmits activation of PAR-2 (260).

PAR activation causes an up-regulation of inflammatory molecules, like cytokines, chemokines, growth factors and adhesion molecules. Experiments in healthy human subjects

have shown that recombinant FVIIa results in a 4-fold increase in the concentrations of IL-8 and IL-6 in plasma (261). Activation of PARs transforms ECs into a proinflammatory phenotype, causing vascular permeability and local accumulation of platelets and leucocytes. In VSMCs, PAR activation mediates contraction, proliferation, migration, hypertrophy and modulation of the ECM, thereby contributing to atherosclerosis and hypertension. Platelets exhibit a pivotal role in the cross-talk between coagulation and inflammation, and are activated directly by thrombin or by proinflammatory mediators, such as PAF (230). The platelet P-selectins not only mediate adherence to leukocytes and ECs, but also enhance monocyte TF expression (262). Figure 11 summarizes PAR activation.

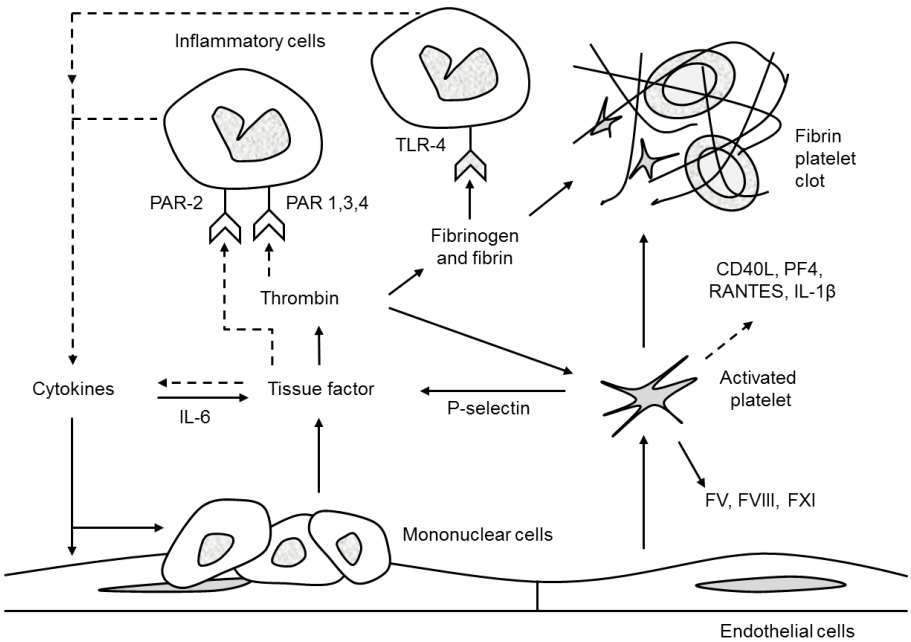

Figure 11. PAR activation. Exposure of TF-bearing cells results in generation of thrombin. Activation of platelets plays a pivotal role. Binding of TF-FVIIa complex or thrombin to specific PARs may affect inflammation by inducing release of cytokines, which modulates coagulation and fibrinolysis. PAR, protease-activated receptors; IL, interleukin; TLR, Toll-like receptor; CD, cluster of differentiation; PF, platelet factor and RANTES, regulated on activation, normal T-cell expressed and secreted. Figure modified from Levi M, 2012 (263).

2 AIMS

Hypertension, FCHL and FH have an increased risk of CVD and premature death. Ang II is implicated in hypertension and promotes atherosclerosis, and patients with hyperlipidaemia may by more sensitive to the potential proinflammatory and procoagulatory effects of Ang II. Antihypertensive treatment may reduce thromboembolic events in hypertension. Thus, the overall aims were investigate the potential importance of Ang II for haemostatic and inflammatory alterations in hypertension and hyperlipidaemia.

The specific aims of this project were as follows:

- To investigate the effects of blocking the RAAS with an ACE inhibitor on blood coagulation in subjects with hypertension (**paper I)**.
- To clarify the impact of antihypertensive treatment by blocking the RAAS on inflammation and haemostasis beyond the effects of BP by comparing the effects of an ACE inhibitor to treatment with an alpha 1-adrenoceptor blocker in subjects with hypertension (**paper II)**.
- To investigate the potential proinflammatory and prothrombotic effects of short-term stimulation by circulating Ang II in healthy individuals (**papers III-V**).
- To study the potential differences in the effects of circulating Ang II on inflammation and haemostasis in patients with FCHL and FH compared to healthy subjects (**papers III-V**).

3 MATERIALS AND METHODS

3.1 PATIENTS AND HEALTHY CONTROLS

None of the patients in **paper I-V** had any history or evidence of CVD, congestive heart failure, diabetes mellitus, atrial fibrillation/flutter, autoimmune disorder, liver or chronic kidney disease.

3.1.1 Papers I and II

3.1.1.1 Study population and procedures, paper I

Subjects with hypertension were included if their diastolic BP was 95-115 mmHg without antihypertensive treatment the previous 4 weeks. Details about the study, presenting the result that ACE inhibitors reduced cardiac workload during stressful situations, have been presented previously (264). In brief, 6 women and 10 men with mean age 51 years and mean body mass index 26 kg/m^2 (range 21-35 kg/m^2) were included. During the study, medication that might affect the coagulation or BP was not allowed. The participants had stage I hypertension according to the WHO classification, with no signs of target organ damage or characteristics that could indicate secondary hypertension. The duration of the hypertension was on average 10 years (range 6-13 years).

The participants were given 5 mg ramipril or placebo once daily, during 6 weeks, in a double-blind cross-over design. All participants then continued into an open treatment with ramipril during a 6-month period. During this phase, dose titration of ramipril was allowed once daily. The goal was to achieve a diastolic BP lower than 95 mmHg, and the mean dose of ramipril was finally 7.5 mg once daily. Testing in the laboratory was carried out at 3 times; at 6 and 12 weeks, and after ramipril treatment at 6 months. The participants arrived to the laboratory at 12 AM. They were instructed to take their study medication at 08-09 AM in order to reach an estimated peak plasma drug concentration during testing. They were instructed to avoid smoking and refrain from caffeine products during the morning.

Blood was collected from antecubital veins into test tubes containing 0.13 mmol/L sodium citrate immediately before and after each mental stress test. The blood samples were then without delay centrifuged at 2000g for 20 minutes at 20°C, and stored at -80°C until analysis.

3.1.1.2 The mental stress test

In **paper I** patients underwent a mental stress test that was a modified video version of Stroop's colour word conflict test during 20 minutes (264). During this test colour words were rapidly shown in incongruent colours, at the same time, a third incongruent colour word was presented orally. The participants were instructed to mark the colour on a protocol with randomly listed colour words and to ignore the other two conflicting information pieces. In this test a hemodynamic response will reach a steady state within 8-10 minutes and the responses are reproducible. After the mental stress test followed a strict 30 minutes of quiet

seated rest. Heart rate and BP were measured by a noninvasive technique (Dinamap Exercise Monitor (Critkon Inc., Tampa, FL).

3.1.1.3 Study population and procedures, paper II

Patients were included if their office systolic BP was >140 mm Hg and/or their diastolic BP was >90 mm Hg. They were randomized to double-blind treatment (stratified by sex) with ramipril 5 mg once daily or doxazosin 4 mg once daily for 2 weeks. During the initial phase, the dose was titrated to ramipril 10 mg or doxazosin 8 mg once daily. Thereafter the participants were treated during an additional 10 weeks. Prior treatment with RAAS blockade was not allowed. We ruled out secondary hypertension by routine biochemical and physical examinations. Patients with WHO stage III hypertension were not included.

We randomized 71 patients (63 were previously never treated for hypertension). In all, 5 women and 5 men discontinued due to reported side effects (8 on doxazosin and 2 on ramipril). One female patient in the ramipril group and one male patient in the doxazosin group were excluded because of difficulties to obtain blood samples. Thus, 59 patients, achieved the targeted 10 weeks of treatment with 8 mg doxazosin once daily or 10 mg ramipril once daily (table 3).

Table 3. Patient characteristics and and demographic information in **paper II**.

	ramipril	doxazosin
Male/female (n)	20/12	19/8
Age (years)	54 ± 13	53 ± 11
Smokers (n)	2	2
Body mass index (kg/m^2)	26 ± 4	27 ± 5
Glucose (mmol/L)	5.2 ± 0.5	5.5 ± 0.4
Office systolic BP (mm Hg)	155 ± 9	151 ± 8
Office systolic BP (mm Hg)	93 ± 7	93 ± 10
Creatinine (µmol/L)	75 ± 15	77 ± 12
eGFR (mL/minute/1.73m^2)	93 ± 15	92 ± 13
Leucocyte count (10^9/L)	5.2 ± 1.4	5.0 ± 1.0
Platelet count (10^9/L)	214 ± 43	219 ± 50
Total cholesterol (mmol/L)	5.3 ± 0.8	5.5 ± 1.3
HDL cholesterol (mmol/L)	1.3 ± 0.4	1.4 ± 0.4
LDL cholesterol (mmol/L)	3.5 ± 0.8	3.5 ± 1.1
Triglycerides (mmol/L)	1.1 ± 1.0	1.1 ± 0.8

Data are presented as mean ± values SD. Blood samples were taken fasting. No significant differences existed between the ramipril and doxazosin groups. BP, blood

pressure values obtained in the office on inclusion; eGFR, estimated glomerular filtration rate, which was calculated using the chronic kidney disease epidemiology collaboration formula; HDL, high density lipoproteins; and LDL, low density lipoproteins

After fasting overnight the participants arrived in the morning for the examinations at baseline and at week 12. The patients were asked to take their study medication 2 h before they arrived to the laboratory, to achieve peak plasma concentrations. They were instructed not to smoke and to refrain from caffeine-containing beverages, fruit juices or vitamin C during the morning, and to refrain from any other medication (including thrombocyte inhibitory drugs for 7 days and non-steroid anti-inflammatory drugs for 2 days) prior the examinations. Fasting blood samples were obtained by blood collection needles (Eclipse, 21G x 1-1/4") after 20 minutes of supine rest into Vacutainer tubes (Becton Dickinson Co. Cedex, Meylan, France).

3.1.2 Paper III-V

3.1.2.1 Study population

In **paper III** and **IV**, FCHL was thought to exist if the lipoprotein phenotypes IIa, IIb, or IV, according to the Fredrickson classification (22), were found in the family or in the patient at several different times. There were 5 patients on lipid lowering therapy with statins and 1 was on antihypertensive treatment with an ACE inhibitor (table 4).

Table 4. Patient characteristics of participants in **paper III** and **IV**.

	FCHL	$Control_{FCHL}$
Male/female (n)	11/5	9/7
Age (years)	47 ± 6	45 ± 6
Smokers (n)	5	2
Body mass index (kg/m^2)	27 ± 3*	25 ± 4
Systolic blood pressure (mm Hg)	130 ± 13	123 ± 12
Diastolic blood pressure (mm Hg)	84 ± 8	81 ± 8
Pulse pressure (mm Hg)	46 ± 13	42 ± 7
Heart rate (beats per minute)	64 ± 9	58 ± 10
Glucose (mmol/L)	5.2 ± 0.9	4.8 ± 0.4
Total cholesterol (mmol/L)	8.4 ± 2.3****	4.7 ± 0.6
HDL cholesterol (mmol/L)	0.8 ± 0.2***	1.3 ± 0.5
LDL cholesterol (mmol/L)	3.4 ± 1.8	3.1 ± 0.6
Triglycerides (mmol/L)	7.4 ± 5.7****	0.8 ± 0.3

Data are presented as mean ± values SD. Statistical evaluation was performed by Student's *t*-test. Significant differences between FCHL and $control_{FCHL}$ are denoted as; *P

< 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. FCHL, familial combined hyperlipidaemia. HDL and LDL, high and low density lipoproteins.

In **paper IV**, we characterized the FCHL patients in **paper III** with respect to insulin resistance by HOMA-IR (calculated as fasting insulin in mU/L *x* glucose in mmol/L/22.5). Placebo experiments with saline infusion were added to verify the stability of the experimental design and to assess the potential influence of diurnal variations (table 5).

Table 5. Patient characteristics in **paper IV**.

	FCHL	$Control_{FCHL}$	Placebo
Male/female (n)	11/5	9/7	4/4
Insulin (mU/L)	10.0 (5.9-12.2)****	3.6 (3.0-4.4)	3.9 (3.1-6.6)
Glucose (mmol/L)	5.2 ± 0.9	4.8 ± 0.4	5.0 ± 0.3
HOMA-IR	2.2 (1.3-3.0)****	0.7 (0.6-0.9)	0.8 (0.6-1.4)

Data are presented as mean values ± SD or as median and interquartile ranges. Statistical evaluation was performed by Student's *t*-test or Mann-Whitney non-parametric test. Significant differences between FCHL and $control_{FCHL}$ are denoted as; *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. FCHL, familial combined hyperlipidaemia and HOMA-IR, Homeostasis model assessment of insulin resistance.

In **paper V** a diagnosis of FH was based on high total cholesterol and LDL cholesterol levels combined with either a family history of high levels of cholesterol of first degree relatives and/or early ischaemic heart disease, or tendon xanthomas at physical examination. There were12 FCHL patients treated with statins and 3 of them were also treated with the cholesterol uptake inhibitor ezetimibe (table 6).

3.1.2.2 Healthy controls

Age-matched healthy control subjects were recruited from the local area by advertisements and from hospital staff.

In **paper III** and **IV** the healthy subjects (9 males and 7 females) had fasting plasma triglyceride levels less than 2.3 mmol/L and total cholesterol levels less than 6.0 mmol/L. Systolic and diastolic BPs were less than 130 and 80 mm Hg, respectively. All healthy controls were free of medicines, including oral contraceptives.

In **paper V** the healthy subjects (8 males and 8 females) had fasting plasma triglyceride levels less than 1.6 mmol/L and total cholesterol levels less than 5.6 mmol/L. Systolic and diastolic BPs were less than 140 and 85 mm Hg, respectively. All healthy controls were free of medicines, including oral contraceptives.

Table 6. Patient characteristics in **paper V**.

	FH	$Control_{FH}$	Placebo
Male/female (n)	8/8	8/8	4/4
Age (years)	43 ± 8	39 ± 10	40 ± 9
Current/former smokers (n)	0/5	0/7	0/3
Body mass index (kg/m^2)	27 (24-29)	24 (21-27)	24 (22-26)
Systolic BP (mmHg)	127 ± 14*	116 ± 12	116 ± 13
Diastolic BP (mmHg)	75 ± 8	73 ± 8	69 ± 7
Pulse pressure (mm Hg)	48 (44-64)**	42 (36-48)	41 (40-52)
Heart rate (beats/minute)	64 ± 11	63 ± 13	56 ± 7
Glucose (mmol/L)	5.0 ± 0.4	5.2 ± 0.4	5.0 ± 0.3
Insulin (mU/L)	6.4 (5.2-7.6)	4.6 (2.8-6.6)	3.9 (3.1-6.6)
HOMA-IR	1.5 (1.1-1.7)	1.0 (0.6-1.6)	0.8 (0.6-1.4)
Total chol (mmol/L)	8.6 ± 1.8****	4.4 ± 0.7	5.1 ± 0.6
HDL chol (mmol/L)	1.0 ± 0.3	1.2 ± 0.4	1.3 ± 0.2
Non-HDL chol (mmol/L)	7.5 ± 1.9****	3.2 ± 0.8	3.9 ± 1.4
LDL chol (mmol/L)	6.8 ± 1.8****	2.8 ± 0.8	3.4 ± 1.1
Triglycerides (mmol/L)	1.3 (0.9-1.8)***	0.7 (0.5-1.0)	0.9 (0.6-1.2)

Data are presented as mean values ± SD or as median and interquartile ranges. Statistical evaluation was performed by Student's *t*-test or Mann-Whitney non-parametric test. Significant differences between FH and $control_{FH}$ are denoted as; *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. BP, blood pressure; HOMA-IR, Homeostasis model assessment of insulin resistance; chol, cholesterol; HDL, High-density lipoprotein and LDL, low-density lipoprotein.

3.1.2.3 Study procedures and blood sampling

All participants on antihypertensive or lipid lowering therapy were instructed to quit their medication 4 weeks before the start of the study. The subjects were asked to avoid smoking on the day of the investigation and avoid taking non-steroid anti-inflammatory or aspirin drugs at least 7 days prior to the investigation. After an overnight fast, the participants arrived at the Cardiovascular Research Laboratory between 07.00 and 08.00 a.m. For the Ang II infusion, an indwelling catheter was applied in supine position in a vein of the left arm. Blood was collected by Vacutainer technique, using blood collection needles (Eclipse, 21G x 1-1/4") inserted in a vein of the right arm. The participants rested in the supine position during 20-30 minutes before the investigations started. BP was measured using a mercury sphygmomanometer technique. BP values are presented as the calculated mean of two separate measurements, taken about one minute apart.

Blood samples were taken at baseline, at 1 and/or 3 h, and 1 h after the infusion of Ang II. Blood was collected into Vacutainer tubes (Becton Dickinson, Meylan, France) that contained EDTA or sodium citrate (3.8%), as appropriate. The blood samples were immediately centrifuged at 2000g, at 20°C for 20 minutes, and then separated and administered in aliquots (0.5 mL) and stored in -80°C until analysis.

3.1.2.4 Ang II infusion

In **paper III** and **IV** we used Ang II (Angiotensin amid, Apoteksbolaget, Umeå, Sweden) while we used Ang II acetate (Clinalfa basic, Bachem AG, Basel, Switzerland) in **paper V**.

Ang II was dissolved in physiological saline. The starting dose was 2 ng/kg/minute and every 5 minutes the dose was incremented with 2 ng/kg/minute up to 10 ng/kg/minute. This dose was then maintained for 3 h. We have used the dose of 10 ng/kg/minute earlier (13) and this dose has been shown to increase plasma Ang II concentrations to about 50 pmol/L, which has been estimated to be equal to 5 to 10 times basal levels in healthy subjects (265). If mean arterial pressure levels increased from the levels before the infusion began by more than 25 mm Hg, the infusion rate was reduced in steps of 2 ng/kg/minute due to a decision from the Regional Ethics Committee.

3.1.2.5 Placebo infusion

In **paper IV** and **V** we added separate experiments with placebo infusion (physiological saline) in eight subjects (4 women) to check the stability of the experimental design and to assess the potential influence of diurnal variations. The placebo participants had fasting triglyceride levels less than 2.2 mmol/L and total cholesterol levels less than 7.1 mmol/L, respectively, and their systolic BP and diastolic BP levels were less than 120 and 80 mm Hg, respectively.

3.2 METHODS

3.2.1 Calibrated automated thrombogram

In **paper II** and **V** thrombin generation potential was measured according to the method calibrated automated thrombogram (CAT), reported by Hemker et al., and in accordance to the manufacturer instructions (Thrombinoscope BV, Maastricht, the Netherlands) (266). We used Thrombinoscope BV reagents.

The reactions were carried out in 96-well microtiter plates (Immulon 2HB transparent U-bottom from Thermo Electron, Denmark). Two wells were needed for each experiment, one well to measure thrombin generation of a plasma sample and another for calibration. Briefly, 80 µl platelet-poor plasma was mixed with 20 µl of a platelet poor-plasma reagent containing TF and phospholipids reaching final concentrations of TF of 5 pM and phospholipids of 4 µM. In addition, 80 µl of the same platelet-poor plasma reagent was mixed with a thrombin calibrator. To start the reactions in wells for calibration and measurement, a fluorogenic

substance, specifically cleaved by thrombin, was then added together with $CaCl_2$ in Hepes buffer.

The fluorescence was analysed every ½ minute during 1 h by a Fluoroscan Ascent fluorometer (Thermo Scientific Vanta, Finland). A commercial software by the manufacturer (Thrombinoscope version 2007) calculated and presented five variables (266):

- Peak thrombin concentration. The peak concentration of thrombin generated
- Endogenous thrombin potential. The area under the curve that corresponds to the total amount of thrombin generated over 1 h.
- Lag-time. The time interval until the beginning of thrombin generation. Corresponds to the clotting time
- Time to peak thrombin concentration. The time interval until the peak thrombin concentration. Takes into account the amplification and propagation phases
- Time to tail. Time that elapsed until the end of the thrombin generation, i.e. inhibition of thrombin generation by various anticoagulants.

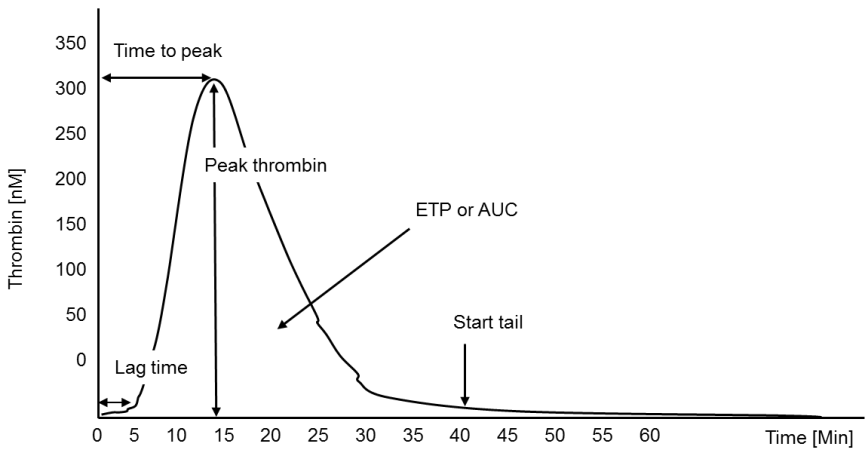

Figure 12. The five variables calculated in calibrated automated thrombogram, ETP, endogenous thrombin potential and AUC, area under curve.

For example, a hypocoagulability state is characterized by a prolonged lag-time, and reductions in both peak thrombin and endogenous thrombin potential. On the other hand, a hypercoagulability state is characterized by a reduced lag-time and increased peak thrombin and endogenous thrombin potential values.

3.2.2 Other laboratory methods

We used commercially kits and calibrators to analyse quantities of inflammatory and haemostatic markers.

3.2.2.1 Paper I

Fibrinogen was measured by a polymerization time method. TAT complex was analysed by using an enzyme immunoassay (Enzygnost TAT Micro; Behringwerke AG, Marburg, Germany). FVII was detected by an amidolytic method measuring all FVII activity generated after thromboplastin activator addition to the test tubes.

3.2.2.2 Paper 2

TAT complex was determined by using an enzyme immunoassay (Enzygnost TAT Micro; Behringwerke AG, Marburg, Germany). PAI-1 activity was analysed by using an enzyme immunoassay (TriniLIZE PAI-1 activity, Tcoag Ireland Ltd., Ireland). T-PA antigen was determined by using assays from R&D Systems (Abingdon, UK). IL-6, IL-8, IL-6R, high sensitive (hs) CRP, TNF-α and MCP-1 were analysed by using assays from MesoScale Diagnostic (Human Cytokine Assay, Ultra-Sensitive Kit, MSD, Bethesda, USA).

3.2.2.3 Paper III

HsIL-6, TNF-α and t-PA/PAI-1 complexes were assessed by using assays from R&D Systems (Abingdon, UK). F1+2 and TAT complex concentrations were determined by using enzyme assays from Behring-Werke AG (Marburg, Germany).

3.2.2.4 Paper IV

PAI-1 activity was analysed by an assay from Hyphen BioMed (Neuville-sur-Oise, France). PAP complex was measured by a classical two-site ELISA (267).

3.2.2.5 Paper V

PAI-1 activity was analysed by using an assay from Hyphen BioMed (Neuville sur Oise, France). PAP complex was determined by a classical two-site ELISA (267). F1+2 concentrations were analysed by using assays from Siemens Healthcare (Marburg, Germany). HsIL-6 was analysed by using assays from R&D Systems (Abingdon, UK). Fibrinogen levels were determined by means of a Fibri-Prest Automate method (von Clauss method) from Diagnostica Stago (Asneres, France).

3.2.2.6 Routine analyses

3.2.2.6.1 Paper II

Leukocyte counts were analysed by using an automated blood cell counter (Technicon H1, Hematology System, Technicon Instruments Corp. Tarrytown, NY, USA). Plasma glucose was determined by an automated routine method (Synchron LX, Beckman Coulter, Inc., Fullerton, CA, USA). The cholesterol and triglyceride levels were analysed by standard enzymatic techniques (Boehringer-Mannheim, Mannheim, Germany).

3.2.2.6.2 Paper III

Leukocyte counts were determined by using an automated blood cell counter (Technicon H1, Hematology System, Technicon Instruments Corp. Tarrytown, NY, USA). Plasma glucose was determined by an automated routine method (Synchron LX, Beckman Coulter, Inc., Fullerton, CA, USA). The various lipoprotein particles were analysed by precipitation and centrifugation steps. The cholesterol and triglyceride levels were analysed by standard enzymatic techniques (Boehringer-Mannheim, Mannheim, Germany).

3.2.2.6.3 Paper IV

HsCRP was determined by turbidimetry (Beckman Coulter, Fullerton, California, USA). Insulin levels were determined by an automated immunometric sandwich method (Modular E170; Roche Diagnostics GmbH, Mannheim, Germany) and detection with electrochemiluminescence immunoassay. Insulin resistance was assessed by the homeostasis model assessment of insulin resistance (HOMA-IR, by the formula; insulin in mU/L × glucose in mmol/L/22.5).

3.2.2.6.4 Paper V

Leukocyte counts and hsCRP count were analysed by using an automated blood cell counter (Technicon H1, Hematology System, Technicon Instruments Corp., Tarrytown, NY, USA. Insulin resistance was assessed by HOMA-IR. Plasma creatinine, glucose, cholesterol and triglyceride contents of the various lipoprotein fractions were assessed by automated standard methods.

3.3 STATISTICAL ANALYSES

Statistical calculations in **papers I-V** were performed using Statistica'99 software, version 7.7, series: 1205 (Statsoft Inc., Tulsa, Oklahoma, USA). Normality was considered to be present if skewness was more than -1 and less than 1. Normally distributed data are presented as mean ± standard deviation (SD), whereas skewed data are presented as median values and interquartile ranges. Variables with skewed distribution were logarithmically transformed. A probability (*P*) less than 0.05 was considered statistically significant.

3.3.1.1 Paper I

We used non-parametric tests, by analysis of variance (ANOVA, Friedman test) with appropriate *post hoc* testing or by paired comparisons (Wilcoxon signed rank test), as appropriate. We estimated that the study, with 2 alpha 0.05, would require 15 participants to offer a power of 0.80 to detect a TAT complex difference of 0.4 µg/L by treatment, with a SD 0.5 µg/L.

3.3.1.2 Paper II

Graphing techniques were used to assess outliers. Extreme values were considered invalid if exceeding 2 SD beyond mean values for the given time and treatment group, as proposed by

Vaughan et al (91), also after logarithmic transformation. For TAT complex, extreme outlier values were present in 5/27 on doxazosin and in 4/32 on ramipril; these values were replaced by the mean for the given time and treatment group. As for the other markers of inflammation and coagulation, only single outliers were identified and excluded. Comparison between study groups and the effects of treatment were made by repeated measures multivariable analysis of variance (MANOVA), accounting for the potential confounding of smoking. Analysis of changes in TAT complex levels at baseline and after 3 months of treatment with ramipril and doxazosin were made by using non-parametric tests by paired comparisons (Wilcoxon signed rank test). Assuming 2 alpha 0.05 and a power of 0.80, we estimated a study population of 2 x 26 subjects in order to detect a 0.4 µg/L difference in TAT complex by treatment, with a SD 0.5 µg/L.

3.3.1.3 Paper III-IV

Changes within and between the groups at baseline were analysed by Student's *t*-test or Mann-Whitney non-parametric test, as appropriate. The responses of Ang II infusion on BP, heart rate, markers of inflammation and haemostasis were determined by repeated measures ANOVA. We estimated that these studies, with 2 alpha 0.05, would require 15 subjects to have a power of 0.80 to detect a TAT complex difference of 0.4 µg/L by infusion of Ang II, with a SD 0.5 µg/L.

3.3.1.4 Paper V

Changes within and between groups at baseline were investigated by Student's *t*-test or Mann-Whitney non-parametric test, as appropriate. The responses of Ang II infusion on BP, heart rate, inflammatory and haemostatic markers were analysed by repeated measures ANOVA. Post hoc calculation indicated that the study, with 2 alpha 0.05, would require 14 subjects to have a power of 0.80 to detect a PAI-1 activity difference of 1.0 ng/mL by Ang II infusion, with a SD 1.3 ng/mL.

3.4 ETHICAL CONSIDERATIONS

The studies were performed in accordance with the Declaration of Helsinki (1989) of the World Medical Association. All studies were approved by the Regional Ethics Committee of Karolinska University Hospital in Stockholm. We received informed consent from all participants.

4 RESULTS

4.1 PAPER I

4.1.1 Effects on blood pressure and heart rate

The results are presented in table 7. Systolic and diastolic BPs were reduced by treatment with ramipril, no effects were observed on heart rate. Placebo during 6 weeks did not affect BP or heart rate (data not shown). Systolic BP and heart rate responses during mental stress were attenuated by ramipril, for details, see (264).

Table 7. Effects of ramipril treatment at rest and during mental stress.

	Placebo	Ramipril, 6 w	Ramipril, 6 m
Blood pressure (mm Hg) and heart rate (beats per minute) at rest			
Systolic BP	154 (138-165)	146 (128-154)*	140 (131-165)
Diastolic BP	106 (94-110)	98 (86-109)***	94 (87-103)**
Heart rate	71 (66-73)	72 (66-80)	73 (67-78)

Data are presented as median values and interquartile ranges during rest and following 20 minutes of mental stress (n=15-16). Statistical evaluation was made by using non-parametric tests by paired comparisons (Wilcoxon signed rank test). Significant differences are given as; *P < 0.05, **P < 0.01, ***P < 0.001 compared to placebo. W, weeks; m, months and BP, blood pressure.

4.1.2 Effects on coagulation

The results are presented in table 8 and figure 13. Ramipril treatment reduced TAT complex after 6 weeks and 6 months, while ramipril tended to reduce fibrinogen levels at 6 months (P=0.06). Ramipril did not affect Factor VII.

Table 8. Effects of ramipril treatment at rest and during mental stress.

	Placebo	Ramipril, 6 w	Ramipril, 6 m
TAT complex (µg/L)			
Rest	2.30 (1.65-3.78)	2.00 (1.38-2.15)*	1.80 (1.62-1.98)*
Stress	2.05 (1.30-2.80)	1.90 (1.50-2.55)	1.80 (1.60-2.55)
Fibrinogen (g/L)			
Rest	3.20 (2.85-4.10)	2.95 (2.80-4.05)	3.00 (2.65-3.42)
Stress	3.45 (3.00-4.10)†	3.50 (3.05-3.90)†	3.10 (2.80-3.35)
Factor VII (mg/L)			
Rest	1.24 (1.08-1.34)	1.24 (1.02-1.48)	1.33 (1.07-1.46)
Stress	1.17 (1.02-1.40)	1.25 (1.10-1.50)	1.14 (1.06-1.40)

Data are presented as median values and interquartile ranges during rest and following 20 minutes of mental stress (n=15-16). Statistical evaluation was made by using non-parametric tests, by ANOVA (Friedman's test) or by paired comparisons (Wilcoxon signed rank test). Significant differences are given as; *P < 0.05 compared to placebo; †P < 0.05 compared to resting conditions. W, weeks; m, months and TAT, thrombin-antithrombin.

Mental stress did not affect TAT or Factor VII. Fibrinogen increased during stress following both placebo and ramipril treatment for 6 weeks, but no effects after 6 months of ramipril treatment.

Figure 13 illustrates changes in TAT complex levels for placebo and after ramipril therapy during 6 weeks and 6 months.

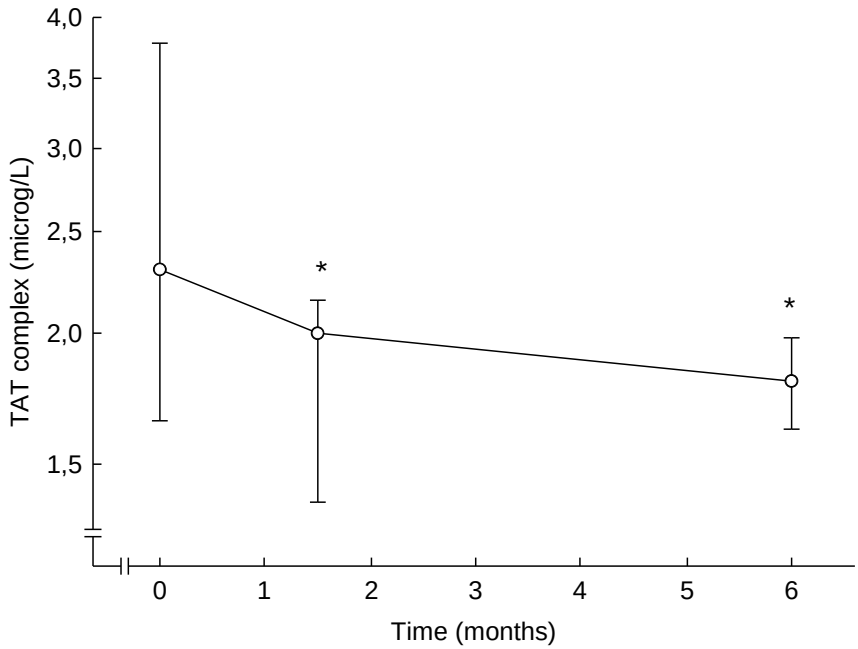

Figure 13. TAT complex at rest and during placebo and during ramipril therapy for 6 weeks and 6 months. Data are presented as median and interquartile ranges, n=15. Significant differences are given as; *P < 0.05, compared to placebo.

4.2 PAPER II

4.2.1 Effects on blood pressure and heart rate

The results are presented in table 9. Antihypertensive treatment reduced systolic and diastolic BP in both study groups.

Table 9. Treatment effects on blood pressure and heart rate.

	Ramipril	Doxazosin	MANOVA
Systolic blood pressure (mm Hg)			
Baseline	155 ± 9	151 ± 8	*P* group=0.38
12 weeks	135 ± 12	142 ± 12	*P* time<0.001
Δ 0 to 12 weeks	-19 ± 12	-11 ± 12	*P* group *x* time=0.23
Diastolic blood pressure (mm Hg)			
Baseline	93 ± 7	93 ± 10	*P* group=0.88
12 weeks	79 ± 6	83 ± 10	*P* time<0.001
Δ 0 to 12 weeks	-15 ± 8	-10 ± 7	*P* group x time=0.085
Heart rate (beats per minute)			
Baseline	62 ± 8	59 ± 8	*P* group=0.65
12 weeks	62 ± 7	59 ± 10	*P* time=0.74
Δ 0 to 12 weeks	0 ± 5	0 ± 7	*P* group *x* time=0.98

Data are given mean values ± SD for 27-32 participants in each treatment group at week 0 or 12. Δ represents absolute changes by treatment given as mean values ± SD.

4.2.2 Effects on inflammation

The results are presented in table 10. Although antihypertensive treatment overall did not affect hsIL-6, there was a difference between treatment with ramipril (increase) and with doxazosin (decrease). There were no changes in the soluble receptor for IL-6, soluble IL-6R, and there were no changes in for the other markers of inflammation, either in treatment with ramipril or doxazosin.

Table 10. Treatment effects on inflammatory markers.

Week	Ramipril	Doxazosin	ANOVA
HsIL-6 (pg/mL)			
0	0.28 (0.21-0.38)	0.29 (0.20-0.39)	*P* group=0.78
12	0.37 (0.24-0.48)	0.26 (0.20-0.43)	*P* time=0.37
Δ 0 to 12	0.13 ± 0.27	-0.01 ± 0.21	*P* group *x* time=0.012

HsIL-6R (ng/mL)			
0	29.2 (24.0-36.4)	30.4 (23.9-37.1)	*P* group=0.97
12	30.0 (24.8-36.1)	29.6 (22.8-36.4)	*P* time=0.58
Δ 0 to 12	0.8 ± 8.4	-2.4 ± 8.6	*P* group x time=0.11
IL-8 (pg/mL)			
0	1.89 (1.34-2.44)	1.78 (1.46-2.17)	*P* group=0.13
12	1.66 (1.33-2.02)	1.45 (1.17-1.82)	*P* time=0.84
Δ 0 to 12	-0.07 ± 0.90	-0.36 ± 0.69	*P* group *x* time=0.69
TNF-α (pg/mL)			
0	1.00 (0.81-1.13)	0.96 (0.80-1.19)	*P* group=0.41
12	1.03 (0.81-1.16)	0.94 (0.82-1.10)	*P* time=0.31
Δ 0 to 12	0.01 ± 0.25	-0.05 ± 0.23	*P* group *x* time0.81
MCP-1 (pg/mL)			
0	42.2 (36.6-48.1)	42.2 (33.8-51.2)	*P* group=0.14
12	41.2 (34.6-43.0)	42.3 (35.5-52-0)	*P* time=0.50
Δ 0 to 12	-0.1 ± 10.6	1.0 ± 9.3	*P* group *x* time=0.90
HsCRP (mg/L)			
0	1.31 (0.65-2.11)	1.80 (1.20-3.04)	*P* group=0.52
12	1.35 (0.70-2.67)	1.38 (0.92-2.25)	*P* time=0.12
Δ 0 to 12	0.13 ± 1.70	0.02 ± 2.34	*P* group *x* time=0.11

Data are given as median values and interquartile ranges for 26-32 participants in each treatment group at week 0 and 12. Δ represents absolute changes by treatment given as mean values ± SD. Hs, high sensitive; IL, interleukin; R, receptor; TNF-α, tumor necrosis factor-α; MCP-1, monocyte chemoattractant protein-1 and CRP, C-reactive protein.

4.2.3 Effects on fibrinolysis

The results are presented in table 11. There were no changes in PAI-1 activity by treatment. Concentrations of t-PA antigen increased by ramipril, while it decreased by doxazosin.

Table 11. Treatment effects on fibrinolytic markers.

Week	Ramipril	Doxazosin	ANOVA
PAI-1 activity (IU/mL)			
0	4.6 (1.2-10.1)	7.1 (2.3-16.1)	*P* group=0.54
12	6.1 (3.0-17.4)	7.2 (2.9-15.1)	*P* time=0.93
Δ 0 to 12	5.3 ± 9.2	-3.2 ± 13.1	*P* group *x* time=0.89

t-PA antigen (ng/mL)			
0	3.49 ± 1.18	3.80 ± 1.08	*P* group=0.94
12	3.67 ± 1.30	3.38 ± 0.96	*P* time=0.38
Δ 0 to 12	0.18 ± 0.55	-0.42 ± 0.62	*P* group x time=0.008

Data are given as mean values ± SD or median values and interquartile ranges for 25-29 participants in each treatment group at week 0 and 12. Δ represents absolute changes by treatment given as mean values ± SD. PAI, plasminogen activator inhibitor and t-PA, tissue plasminogen activator.

4.2.4 Effects on coagulation

The results are presented in figure 14 and table 12. TAT complex decreased by treatment, and this was dependent on a reduction in TAT complex in the ramipril group alone. There were no changes in the CAT data by treatment.

Figure 14 illustrates the changes in TAT complex after 3 months of therapy with ramipril and doxazosin.

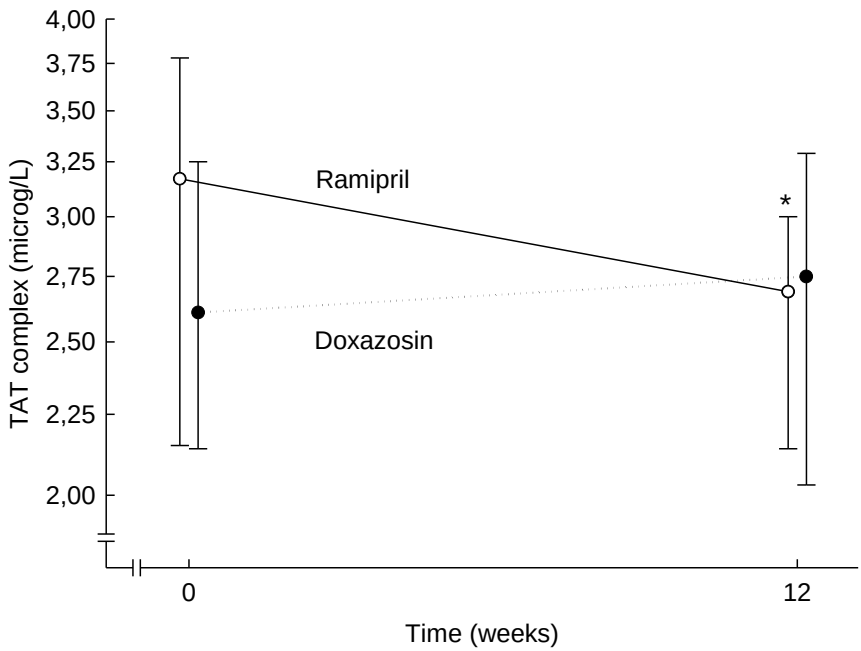

Figure 14. TAT complex levels at baseline and after 3 months of treatment with ramipril and doxazosin. Data are presented as median values and interquartile ranges, n=28 for the ramipril group and n=22 for the doxazosin group. Statistical evaluation was made by using non-parametric tests by paired comparisons (Wilcoxon signed rank test). Significant differences are given as; *$P < 0.05$.

Table 12. Treatment effects on markers of coagulation.

Week	Ramipril	Doxazosin	MANOVA
TAT complex (µg/L)			
0	3.17 (2.15-3.78)	2.61 (2.14-3.23)	*P* group=0.14
12	2.69 (2.14-3.00)	2.75 (2.03-3.29)	*P* time=0,025
Δ 0 to 12	-0.47 ± 1.21	-0.05 ± 0.73	*P* group *x* time=0.014
CAT, peak thrombin (nM)			
0	321 51	322 46	*P* group=0.11
12	313 61	337 29	*P* time=0.92
Δ 0 to 12	-8 ± 71	15 ± 52	*P* group x time=0.45
CAT, endogenous thrombin potential (nM *x* minutes)			
0	1938 ± 298	2071 ± 270	*P* group=0.12
12	1975 ± 396	2068 ± 386	*P* time=0.93
Δ 0 to 12	37 ± 380	-3 ± 323	*P* group *x* time=0.50
CAT, lag-time (minutes)			
0	3.7 ± 0.9	3.8 ± 0.6	*P* group=0.68
12	3.8 ± 0.8	3.5 ± 0.6	*P* time=0.27
Δ 0 to 12	0.1 ± 1.1	-0.3 ± 0.7	*P* group *x* time=0.14
CAT, time to peak (minutes)			
0	6.7 ± 1.4	6.9 ± 1.1	*P* group=0.26
12	6.8 ± 1.2	6.6 ± 1.0	*P* time=0.53
Δ 0 to 12	0.1 ± 1.6	-0.4 ± 1.3	*P* group *x* time=0.44

Data are given as mean values ± SD or as median values and interquartile ranges for 22-31 participants in each treatment group at week 0 or 12. Δ represents absolute changes by treatment given as mean values ± SD. TAT, thrombin-antithrombin and CAT, calibrated automated thrombogram.

Changes in TAT by treatment did not relate significantly to changes in BP from baseline to week 12, i.e., r = -0.01 (p = 0.98) for systolic BP, r = -0.08 (p = 0.60) for diastolic BP and r = 0.04 (p = 0.76) for pulse pressure (n=50); similar relations were obtained when the two study groups were analysed separately.

4.3 PAPER III-V

4.3.1 Effects on blood pressure and heart rate

4.3.1.1 Baseline

The results are presented in tables 4 and 6. **Papers III** and **IV**: FCHL had similar BP and heart rate compared to control$_{FCHL}$. **Paper V**: In FH, systolic BP and pulse pressure were higher compared to control$_{FH}$ ($P < 0.05$ and $P < 0.01$, respectively).

4.3.1.2 Ang II infusion

The results are presented in table 13 and 14. Ang II infusion caused a marked and rapid increase in BP in all groups. **Paper III** and **IV**: In FCHL, the systolic BP increase was greater compared to control$_{FCHL}$. The diastolic BP increased similarly in FCHL and control$_{FCHL}$. After the end of Ang II infusion, BP returned rapidly to baseline levels. The heart rate decreased in both groups and returned to baseline levels after the end of the infusion. The heart rate responses were similar in FCHL and control$_{FCHL}$.

Table 13. Systolic and diastolic blood pressure and heart rate before, during and after angiotensin II infusion in FCHL and control$_{FCHL}$.

	0 h	0.5 h	2.5 h	+0.5 h	ANOVA
Systolic blood pressure (mm Hg)					
FCHL	130 ± 13	161 ± 19	160 ± 11	131 ± 13	*P* group=0.002
Control$_{FCHL}$	123 ± 12	143 ± 10	142 ± 11	119 ± 11	*P* time<0.0001
					P group *x* time=0.003
Diastolic blood pressure (mm Hg)					
FCHL	84 ± 8	103 ± 7	102 ± 9	82 ± 8	*P* group=0.028
Control$_{FCHL}$	81 ± 8	96 ± 9	95 ± 8	76 ± 9	*P* time<0.0001
					P group *x* time=0.136
Heart rate (beat per minute)					
FCHL	64 ± 9	62 ± 7	61 ± 7	67 ± 10	*P* group=0.043
Control$_{FCHL}$	58 ± 10	56 ± 8	56 ± 8	61 ± 9	*P* time<0.0001
					P group *x* time=0.944

Data are presented as mean values ± SD, n=16 for each group. Comparisons over time were evaluated by repeated measures ANOVA. FCHL, familial combined hyperlipidaemia.

Paper V: In FH, systolic and diastolic BP increased during Ang II infusion, and the responses were similar in FH compared to control$_{FH}$. After the end of Ang II infusion, BP returned rapidly to baseline levels. The heart rate decreased in both groups, and returned

to baseline levels after the infusion. The heart rate responses were similar in FH and $control_{FH}$.

Table 14. Systolic and diastolic blood pressure and heart rate before, during and after angiotensin II infusion in FH, $control_{FH}$ and saline (placebo).

	0 h	0.3 h	3 h	+0.3 h	ANOVA
Systolic blood pressure (mm Hg)					
FH	127 ± 14	152 ± 18	152 ± 13	127 ± 14	*P* group<0.01
$Control_{FH}$	116 ± 12*	139 ± 16	138 ± 14	117 ± 10	*P* time<0.0001
					P group *x* time=0.78
Placebo	112 ± 9	107 ± 11	111 ± 10	111 ± 8	*P* time=0.60
Diastolic blood pressure (mm Hg)					
FH	74 ± 8	99 ± 7	93 ± 8	77 ± 10	*P* group=0.11
$Control_{FH}$	73 ± 8	90 ± 11	88 ± 10	70 ± 10	*P* time<0.0001
					P group *x* time=0.10
Placebo	69 ± 7	71 ± 12	73 ± 13	69 ± 7	*P* time=0.70
Heart rate (beat per minute)					
FH	64 ± 11	61 ± 14	62 ± 11	66 ± 11	*P* group=0.75
$Control_{FH}$	63 ± 13	59 ± 12	60 ± 11	67 ± 11	*P* time<0.0001
					P group *x* time=0.39
Placebo	56 ± 7	56 ± 5	57 ± 7	59 ± 7	*P* time=0.60

Data are presented as mean values ± SD, n=16 for FH and for $control_{FH}$ and n=8 for placebo. At baseline, statistical evaluation was performed by Student's *t*-test. Significant differences are given as; $*P < 0.05$. Comparisons over time were evaluated by repeated measures ANOVA. FH, familial hypercholesterolemia.

Due to a limitation from the Regional Ethics Committee, the mean arterial pressure was not allowed to increase by more than 25 mm Hg. Therefore, the Ang II infusion rate was reduced in 6 FCHL and in 2 $control_{FCHL}$, and the maintenance dose of Ang II was lower in FCHL, as compared to $control_{FCHL}$ (9.1±1.0 vs. 9.8±0.7 ng/kg/minute, $P < 0.05$) (**paper III** and **IV)**. In **paper V**, Ang II infusion rate was reduced in 7 FH and in 3 $control_{FH}$. The maintenance doses of Ang II were similar in both FH and $control_{FH}$ (7.5±2.8 vs. 8.7±2.3 ng/kg/minute, P=0.18).

4.3.2 Effects on inflammation

4.3.2.1 Baseline

The results are presented in table 15. **Paper III** and **IV**: FCHL had higher levels of IL-6 ($P < 0.05$), leukocyte counts ($P < 0.001$) and hsCRP ($P < 0.05$) as compared to $control_{FCHL}$, while TNF-α was on the same levels in both groups. **Paper V**: FH had the same levels of IL-6, leukocyte counts, TNF-α and hsCRP compared to $control_{FH}$. However, the fibrinogen level was higher in FH ($P < 0.05$) compared to $control_{FH}$.

Table 15. Markers of Inflammation before, during and after angiotensin II infusion in FCHL, $control_{FCHL}$, FH, $control_{FH}$ and saline (placebo).

	0 h	3 h	+1 h	ANOVA
HsIL-6 (pg/mL)				
FCHL	1.1(1.0-1.8)	2.3(1.6-2.7)	2.9(1.9-3.6)	*P* group=0.035
$Control_{FCHL}$	0.7(0.5-1.2)*	1.5(1.1-2.1)	2.4(1.5-3.4)	*P* time<0.0001
				P group *x* time=0.19
FH	0.4(0.4-0.9)	1.1(0.8-1.5)	2.0(0.8-4.3)	*P* group=0.21
$Control_{FH}$	0.6(0.3-1.0)	1.9(0.9-2.8)	2.5(1.5-4.1)	*P* time<0.0001
				P group *x* time=0.60
Placebo	0.8(0.7-1.4)	1.0(0.6-2.3)	1.2(0.7-2.6)	*P* time=0.10
Leukocyte count (*x* 10^9)				
FCHL	5.9(5.5-6.9)	6.4(5.9-8.0)	6.1(5.6-7.3)	*P* group=0.004
$Control_{FCHL}$	4.6(3.9-5.5)***	5.5(4.3-7.3)	5.4(4.1-6.6)	*P* time<0.0001
				P group *x* time=0.03
FH	5.2 ± 1.2	5.7 ± 1.4	5.8 ± 1.5	*P* group=0.38
$Control_{FH}$	5.6 ± 1.3	6.4 ± 1.7	6.1 ± 1.6	*P* time<0.0001
				P group *x* time=0.30
Placebo	5.5 ± 0.6	5.6 ± 0.8	5.8 ± 1.1	*P* time=0.47
TNF-α (pg/mL)				
FCHL	1.0 ± 0.3	1.1 ± 0.2	1.1 ± 0.2	*P* group=0.90
$Control_{FCHL}$	1.0 ± 0.4	1.0 ± 0.3	1.1 ± 0.3	*P* time=0.17
				P group *x* time=0.42
HsCRP (mg/L)				
FCHL	1.7(0.8-3.6)			
$Control_{FCHL}$	0.6(0.3-2.1)*			
FH	1.5(0.9-2.8)			
$Control_{FH}$	0.5(0.2-2.0)			
Placebo	0.6(0.3-1.3)			

Fibrinogen (g/L)			
FH	2.7(2.2-3.1)	3.0(2.4-3.2)	*P* group=0.004
$Control_{FH}$	2.1(2.0-2.4)*	2.2(2.0-2.4)	*P* time=0.005
			P group *x* time=0.68
Placebo	2.1(1.9-2.3)	1.9(1.9-2.5)	*P* time=0.67

Data are presented as mean values ± SD or as median values and interquartile ranges. At baseline, statistical evaluation was performed by Student's *t*-test or Mann-Whitney non-parametric test. Significant differences are given as; **P* < 0.05, ***P* < 0.01 or ****P* < 0.001. Comparisons over time were evaluated by repeated measures ANOVA. FCHL, familial combined hypercholesterolemia and FH, familial hypercholesterolemia.

4.3.2.2 Ang II infusion

The results are presented in table 15 and figure 15. **Paper III-V**: Ang II infusion caused a similar increase in IL-6 in all four groups. The leukocyte count increased in a similar manner in all four groups during Ang II infusion. TNF-α did not change either in FCHL or $control_{FCHL}$. Fibrinogen increased in $control_{FH}$ (*P* time < 0.05) and tended to increase in FH (*P* time=0.07). We could not observe any changes in placebo.

Figure 15 illustrates the changes in IL-6 in all groups during Ang II infusion.

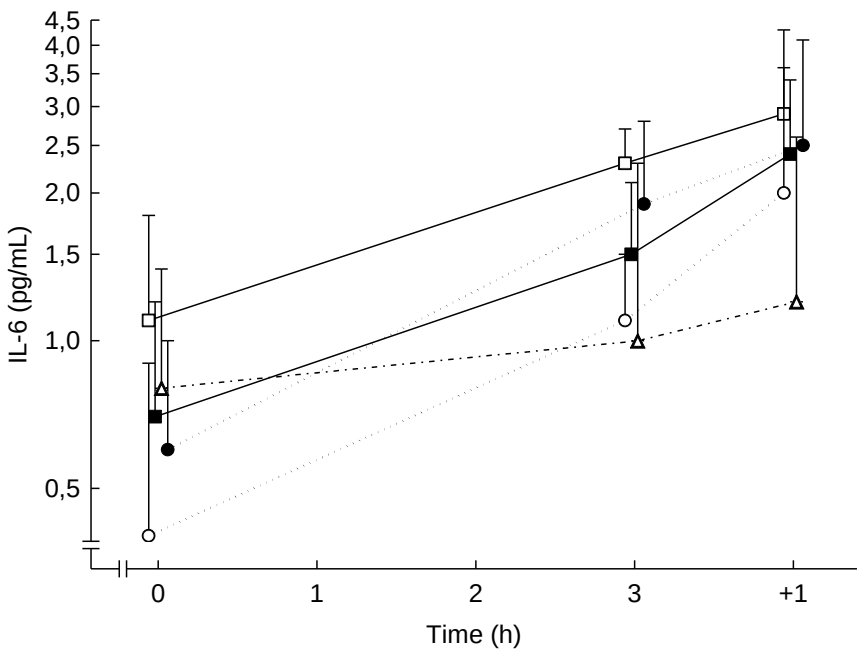

Figure 15. IL-6 in plasma before, during and after angiotensin II infusion. Data are presented as median values and interquartile ranges. PAI-1, plasminogen activator inhibitor-1; FCHL, familial combined hyperlipidaemia and FH, familial

hypercholesterolemia. FCHL, □; $control_{FCHL}$, ■; FH, ○ and $control_{FH}$, ●. The effect of physiological saline infusion in placebo (Δ) is also shown.

4.3.3 Effects on fibrinolysis

4.3.3.1 Baseline

The results are presented in table 16. **Paper III** and **IV**: FCHL had 3-fold higher levels of both t-PA/PAI-1 complexes and PAI-1 activity compared to $control_{FCHL}$ (both $P < 0.001$). Also PAP complex were higher in FCHL compared to $control_{FCHL}$ ($P < 0.001$). **Paper V**: In FH, PAI-1 activity and PAP complex levels were similar to $control_{FH}$.

Table 16. Markers of fibrinolysis before, during and after angiotensin II infusion in FCHL, $control_{FCHL}$, FH, $control_{FH}$ and saline (placebo).

	0 h	1h	3 h	+1 h	ANOVA
PAI-1 activity (ng/mL)					
FCHL	3.4 (1.0-7.0)	2.3 (0.8-5.9)	1.0 (0.3-2.3)	0.9 (0.3-1.8)	*P* group<0.0001
$Contr_{FCHL}$	0.6 (0.2-1.1)***	0.5 (0.2-0.8)	0.2 (0.1-0.3)	0.2 (0.1-0.3)	*P* time<0.0001
					P g *x* t=0.07
FH	1.1 (0.6-1.7)	0.5 (0.3-1.3)	0.2 (0.1-0.5)	0.2 (0.1-0.6)	*P* group=0.44
$Contr_{FH}$	0.9 (0.5-2.9)	0.6 (0.4-1.5)	0.4 (0.2-0.6)	0.3 (0.3-0.5)	*P* time<0.0001
					P g *x* t=0.64
Placebo	0.7 (0.5-1.7)	0.4 (0.3-1.1)	0.3 (0.2-0.5)	0.2 (0.1-0.2)	*P* time<0.0001
PAP complex (µg/mL)					
FCHL	157 ± 37	147 ± 34	146 ± 32	146 ± 29	*P* group<0.001
$Contr_{FCHL}$	103 ± 8***	105 ± 8	112 ± 12	127 ± 26	*P* time=0.08
					P g *x* t<0.001
FH	99 ± 13	95 ± 14	106 ± 14	109 ± 15	*P* group=0.99
$Contr_{FH}$	94 ± 27	96 ± 27	110 ± 44	109 ± 41	*P* time<0.0001
					P g *x* t=0.57
Placebo	83 ± 23	85 ± 20	88 ± 13	92 ± 20	*P* time=0.21
t-PA/PAI-1 complex (ng/mL)					
FCHL	3.0 ± 0.8	2.7 ± 0.7	2.6 ± 0.8	2.6 ± 0.8	*P* group<0.0001
$Contr_{FCHL}$	1.0 ± 0.8***	1.0 ± 0.7	0.9 ± 0.7	0.7 ± 0.6	*P* time<0.0001
					P g *x* t=0.07

Data are presented as mean values ± SD or as median values and interquartile ranges. At baseline, statistical evaluation was performed by Student's *t*-test or Mann-Whitney non-parametric test. Significant differences are given as; **P* < 0.05, ***P* < 0.01 or ****P* < 0.001. Comparisons over time were evaluated by repeated measures ANOVA. FCHL, familial combined hypercholesterolemia; FH, familial hypercholesterolemia; $Contr_{FCHL}$,

control FCHL; $Contr_{FH}$, control FH; t-PA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor-1 and PAP, plasmin-antiplasmin.

4.3.3.2 Ang II infusion

The results are presented in table 16 and figures 16-18. **Paper III** and **IV**: FCHL and $control_{FCHL}$ had similar decreases in t-PA/PAI-1 complex (both *P* time < 0.0001) and PAI-1 activity (both *P* time < 0.0001) during Ang II infusion. PAP complex levels remained unchanged in FCHL during infusion of Ang II (*P* time=0.36) and during physiological saline infusion (*P* time=0.21). PAP complex levels increased in $control_{FCHL}$ stimulation by during Ang II (*P* time < 0.0001). **Paper V**: In FH and $control_{FH}$ we observed a decrease in PAI-1 activity (both, *P* time < 0.0001) and an increase in PAP complex (both, *P* time < 0.0001) during Ang II infusion. During saline infusion caused a decrease in PAI-1 activity (*P* time < 0.0001), reflecting a diurnal variation in fibrinolysis.

Figure 16 and 17 illustrates the changes in PAI-1 activity and PAP complex in FCHL, $control_{FCHL}$, FH, $control_{FH}$ and placebo during Ang II infusion, and figure 18 illustrates the changes in t-PA/PAI-1 complex in FCHL and $control_{FCHL}$ during Ang II infusion.

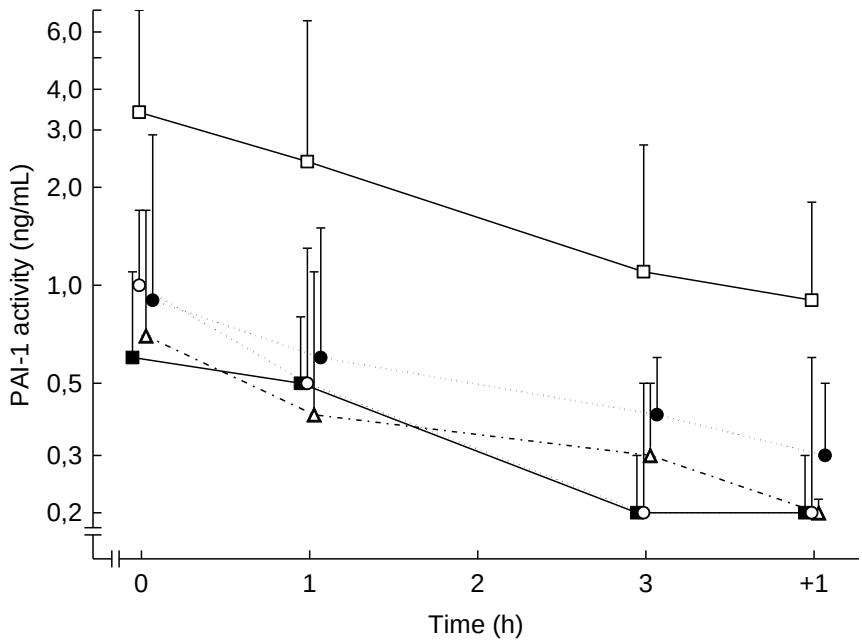

Figure 16. PAI-1 activity in plasma before, during and after angiotensin II infusion. Data are presented as median values and interquartile ranges. PAI-1, plasminogen activator inhibitor-1; FCHL, familial combined hyperlipidaemia; FH, familial hypercholesterolemia. FCHL, □; $control_{FCHL}$, ■; FH, ○ and $control_{FH}$, ●. The effect of physiological saline infusion in placebo experiments (Δ) is also shown.

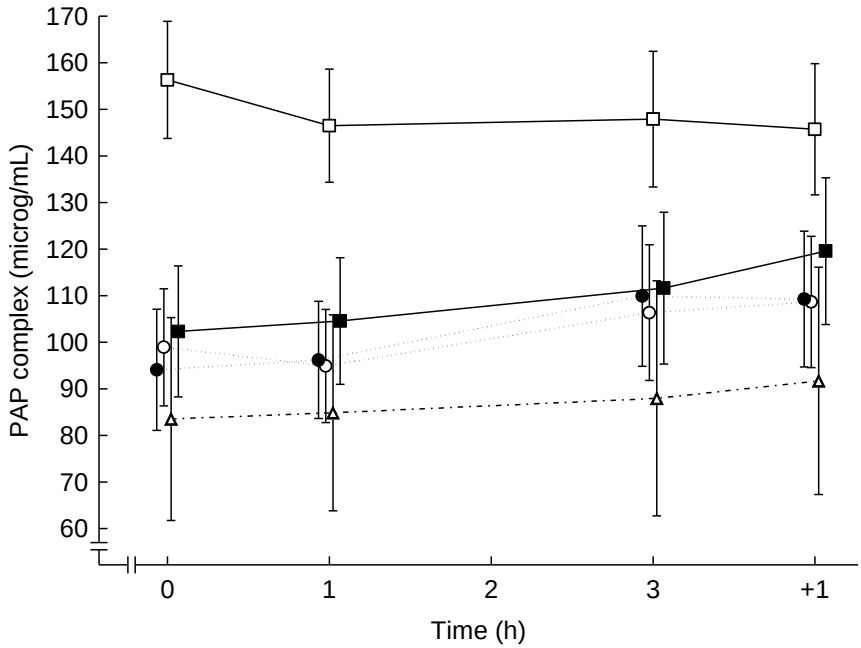

Figure 17. PAP complex in plasma before, during and after angiotensin II infusion. Data are presented as mean values ± SD. PAP, plasmin-antiplasmin; FCHL, familial combined hyperlipidaemia and FH, familial hypercholesterolemia. FCHL, □; $control_{FCHL}$, ■; FH, ○ and $control_{FH}$, ●. The effect of physiological saline infusion in placebo experiments (Δ) is also shown.

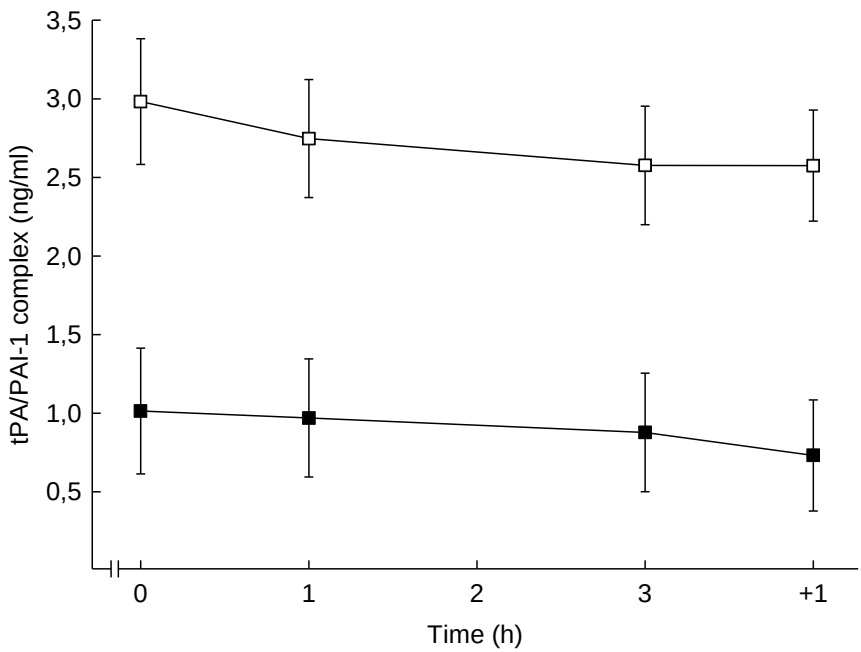

Figure 18. T-PA/PAI complex in plasma before, during and after angiotensin II infusion. Data are presented as mean values ± SD. T-PA, tissue plasminogen activator; PAP, plasmin-antiplasmin and FCHL, familial combined hyperlipidaemia. FCHL, □ and $control_{FCHL}$, ■.

4.3.4 Effects on coagulation

4.3.4.1 Baseline

The results are presented in table 17. **Paper III**: FCHL and $control_{FCHL}$ had the same concentrations of F1+2 and TAT complexes. **Paper V**: FH and $control_{FH}$ had the same F1+2 levels.

Table 17. Coagulation markers before, during, and after angiotensin II infusion in FCHL, $control_{FCHL}$, FH, $control_{FH}$ and saline (placebo).

	0 h	1 h	3 h	+1 h	ANOVA
TAT complex (µg/L)					
FCHL	1.5 (1.3-1.8)	1.8 (1.6-2.5)	2.3 (1.8-3.0)	2.1 (1.5-4.2)	*P* group=0.94
C_{FCHL}	1.7 (1.6-2.6)	1.8 (1.4-2.3)	2.5 (1.6-3.1)	1.7 (1.4-3.4)	*P* time=0.14
					P g *x* t=0.62
FCHL	1.5 (1.3-1.8)	1.8 (1.6-2.5)	2.3 (1.8-3.0)		*P* group=0.77
C_{FCHL}	1.7 (1.6-2.6)	1.8 (1.4-2.3)	2.5 (1.6-3.1)		*P* time=0.03
					P g *x* t=0.38
F1+2 (pmol/mL)					
FCHL	135 ± 36		134 ± 44	130 ± 37	*P* group=0.20
C_{FCHL}	122 ± 29		121 ± 36	110 ± 31	*P* time=0.09
					P g *x* t=0.69
FH	189 (152-229)	174 (139-201)	183 (139-216)	197 (137-222)	*P* group=0.14
C_{FH}	157 (130-226)	169 (97-196)	144 (112-192)	162 (105-187)	*P* time=0.29
					P g *x* t=0.96
Placebo	148 (135-167)	119 (118-133)	122 (112-133)	111 (105-132)	*P* time<0.05

Data are presented as mean values ± SD or as median values and interquartile ranges. At baseline, statistical evaluation was performed by Student's *t*-test or Mann-Whitney non-parametric test. Comparisons over time were evaluated by repeated measures ANOVA. FCHL, familial combined hypercholesterolemia; FH; familial hypercholesterolemia; C_{FCHL}, control FCHL and C_{FH}, control FH.

4.3.4.2 Ang II infusion

The results are presented in table 17 and figure 19. **Paper III**: FCHL and control$_{FCHL}$ had similar, not significant, increases in TAT complex during Ang II infusion when analysing the response in TAT complex during the infusion time, and including 1 h after the end of the infusion. On the other hand, if we analyse TAT complex during the *ongoing* infusion (0 h to 3 h), we instead get a significant increase in TAT complex, similar in FCHL and control$_{FCHL}$. **Paper III-V**: F1+2 did not change in FCHL, control$_{FCHL}$, FH or control$_{FH}$ during Ang II infusion. In placebo, we observed a decrease in F1+2 during saline infusion.

Figure 19 illustrates the changes in TAT complex in FCHL and control$_{FCHL}$ during the *ongoing* Ang II infusion.

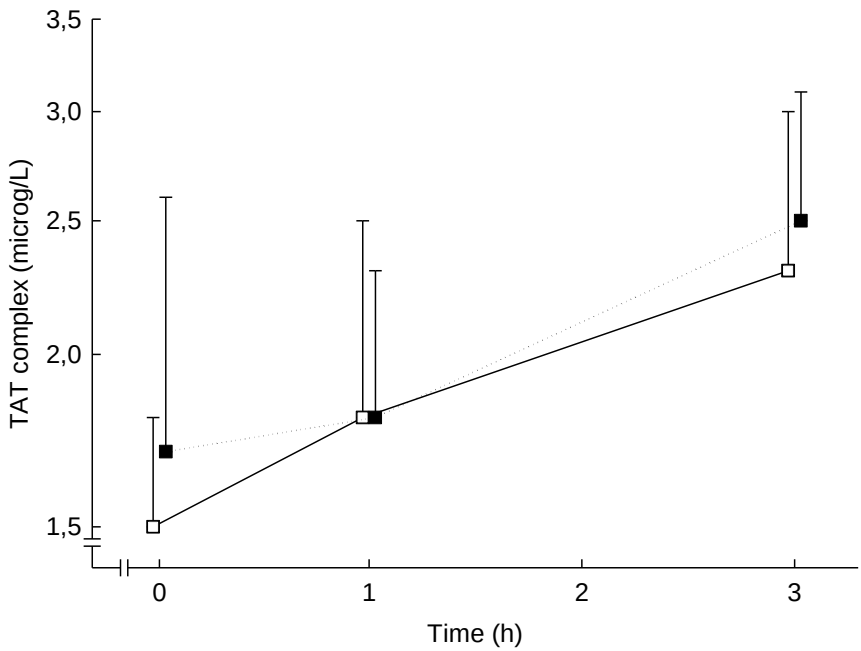

Figure 19. TAT complex in plasma before and during the *ongoing* angiotensin II infusion. Data are presented as median values and interquartile ranges. TAT, thrombin-antithrombin and FCHL, familial combined hyperlipidaemia. FCHL, □ and control$_{FCHL}$, ■.

4.3.4.3 Calibrated automated thrombogram

4.3.4.3.1 Baseline

The results are presented in table 18. **Paper V**: In FH subjects, we observed a higher peak concentration of thrombin generated and a higher endogenous thrombin potential (i.e. total amount of thrombin generated) compared to findings from control$_{FH}$. FH and control$_{FH}$ had similar time dependent parameters of thrombin generation; lag-time, time to peak thrombin and time to tail.

Table 18. Calibrated automated thrombogram parameters before, during and after angiotensin II infusion in FH, control$_{FH}$ and saline (placebo).

	0 h	1 h	3 h	+1 h	ANOVA
Peak thrombin (nM)					
FH	367 ± 47	367 ± 61	368 ± 56	364 ± 64	*P* group<0.05
C_{FH}	311 ± 57**	304 ± 65	303 ± 68	312 ± 56	*P* time=0.73
					P g *x* t=0.89
Pbo	325 ± 40	318 ± 43	314 ± 38	306 ± 48	*P* time=0.59
Endogenous thrombin potential (nM *x* minutes)					
FH	2452 (2065-2768)	2494 (2264-2771)	2478 (2241-2813)	2423 (2151-2790)	*P* group<0.05
C_{FH}	2000 (1821-2194)**	2019 (1776-2176)	1981 (1747-2240)	2028 (1761-2104)	*P* time=0.08
					P g *x* t=0.73
Pbo	2011 (1878-2034)	1957 (1880-2230)	1890 (1867-2312)	1838 (1738-2233)	*P* time=0.37
Lag-time (minutes)					
FH	3.7 ± 0.4	3.8 ± 0.5	3.9 ± 0.4	3.8 ± 0.4	*P* group=0.08
C_{FH}	3.4 ± 0.6	3.5 ± 0.7	3.5 ± 0.7	3.5 ± 0.6	*P* time<0.001
					P g *x* t=0.38
Pbo	4.0 ± 0.6	4.1 ± 0.6	4.1 ± 0.6	4.2 ± 0.6	*P* time=0.16
Time to peak thrombin (minutes)					
FH	6.5 (6.2-7.3)	6.8 (6.2-7.6)	6.8 (6.5-7.7)	6.9 (6.4-7.4)	*P* group=0.33
C_{FH}	6.6 (5.7-7.0)	6.6 (6.0-7.2)	6.6 (6.0-7.3)	6.5 (5.7-6.9)	*P* time<0.01
					P g *x* t=0.28
Pbo	6.8 (6.6-8.2)	7.0 (6.5-8.3)	7.0 (6.3-8.1)	6.7 (6.5-8.1)	*P* time=0.69
Time to tail (minutes)					
FH	26.5 (24.5-29.8)	27.0 (25.2-30.4)	27.7 (25.0-31.0)	27.5 (25.3-30.0)	*P* group=0.22
C_{FH}	25.8 (23.2-27.2)	24.0 (23.1-29.0)	25.0 (24.0-29.0)	24.1 (22.2-28.5)	*P* time=0.10
					P g *x* t=0.46
Pbo	25.0 (23.5-27.5)	25.0 (24.5-27.5)	25.9 (24.5-27.5)	24.0 (23.7-26.5)	*P* time=0.50

FH: Familial hypercholesterolemia. Data are presented as mean values ± SD or median values and interquartile ranges; n=16 for each FH and control$_{FH}$, and n=8 for placebo. At

baseline, statistical evaluation was performed by Student's *t*-test or Mann-Whitney non-parametric test. Significant differences are given as; **P* < 0.05 or ***P* < 0.01. Comparisons over time were evaluated by repeated measures ANOVA. FH, familial hypercholesterolemia. C_{FH}, $control_{FH}$; Pbo, placebo and g *x* t, group *x* time.

4.3.4.3.2 Ang II infusion

The results are presented in table 18. **Paper V**: Neither, peak concentration of thrombin generated or endogenous thrombin potential (i.e. total amount of thrombin generated) did change during infusion of Ang II. We could observe that the thrombin generation was slightly delayed as reflected by increases in the time dependent parameters; lag-time, time to peak and time to tail, and this was due to an increase in FH alone during Ang II infusion, *P* time < 0.01, *P* time < 0.01 and *P* time < 0.05, respectively. No changes were observed in $control_{FH}$ or placebo.

5 GENERAL DISCUSSION

5.1 STUDIES IN HYPERTENSION

5.1.1 Paper I

The main finding in **paper I** was that treatment with the ACE inhibitor ramipril, compared to placebo, decreased thrombin generation in essential hypertension. TAT complex levels, a marker of thrombin generation *in vivo*, were attenuated after 6 weeks of treatment, and the effects were retained after 6 months of therapy. FVII activity levels were unaffected, indicating that other mechanisms than reduced availability of FVII were more likely to be operating. Fibrinogen levels tended to decrease during ramipril treatment at 6 months (P = 0.06). These findings are in agreement with findings in overweight hypertensive patients treated with perindopril (268).

Fibrinogen is an acute phase reactant, as well as a marker of the activity in the coagulation system. Fibrinogen is an independent risk factor of future CHD (269, 270), and high levels of fibrinogen and LDL cholesterol have been reported to be present in plaque formation in vessels (271). Furthermore, VSMC migration and proliferation can be stimulated by fibrinogen suggesting that fibrinogen might be involved in early plaque formation (271-273). Fibrinogen also binds to ICAM-1and can stimulate gene expression of this adhesion molecule, promoting the adhesion of platelets and leukocytes to ECs (274). Hence, fibrinogen mediates inflammation in addition to its central importance in the coagulation cascade.

The attenuated thrombin generation and fibrinogen levels during ramipril treatment could be of importance for the reduction of thromboembolic events seen during treatment with ramipril in patients at high cardiovascular risk (9). However, the antithrombotic effects of ramipril might have been due to the reduction in BP *per se*. To further assess the contribution of BP decrease to the antithrombotic effects we compared ramipril treatment with the alpha 1-adrenoceptor blocker doxazosin in **paper II**.

5.1.2 Paper II

5.1.2.1 Coagulation

Confirming our findings in **paper I**, plasma levels of TAT complex decreased by ramipril therapy in **paper II**. As TAT complex was unaffected in the doxazosin group, and the reductions in BP between the groups were comparable and there was no relation between changes in TAT complex and changes in BP by treatment, the anti-thrombin effects of ramipril are not likely to be related to reduction in BP.

Ang II has been reported to up-regulate expression of the key initiator of coagulation, tissue factor (11), and tissue factor is elevated in hypertensive patients, as compared with normotensive controls (275). Studies in vitro and in vivo have shown that Ang II stimulates formation of procoagulant MPs from ECs and mononuclear cells (276, 277), and that

ACE inhibition reduces monocyte tissue factor expression and activity in plasma in hypertensive patients (12, 278). Our findings of reduced plasma levels of TAT complex following treatment with ramipril is in line with a decreased expression and activity of tissue factor by ACE inhibition, resulting in an attenuated thrombin generation in plasma. This confirms our previous findings (12) and clearly provides one possible mechanism by which ACE inhibition may reduce the risk of atherothrombotic complications in high-risk cardiovascular patients (279, 280).

We observed no changes in CAT data over time in the ramipril or the doxazosin treated groups. CAT measures thrombin generation potential in plasma ex vivo, while TAT complex measures thrombin generation in vivo including effects exerted by the vascular endothelium and blood flow (281). In the present study thrombin generation potential in plasma ex vivo was unaltered. However, as we added exogenous tissue factor and phospholipids to trigger thrombin generation in the plasma samples, the contribution of tissue factor exposing MPs in the patient sample cannot be properly evaluated. Thus, we cannot exclude the possibility that the reduced levels of TAT complex in the ramipril treated group reflect an attenuated thrombin generation which is, at least partly, due to reduced expression of tissue factor on MPs originating from e.g. ECs and/or monocytes. To get a better understanding of such mechanisms, one should measure plasma concentrations of MPs exposing tissue factor, as well as its origins, which could be the focus of future studies.

It is to be noted that other mechanisms may operate to reduce the levels of thrombin generation by ramipril that we observed in **papers I** and **II**. Natural inhibitors such as AT, the PC system or TFPI may contribute to the attenuated levels of TAT complex observed.

In conclusion, in **papers I** and **II** antihypertensive treatment with ramipril suggest reduced thrombin generation beyond the effects on BP reduction alone. Thus, drugs blocking the RAAS may reduce atherothrombotic complications beyond their effects to reduce BP. Whether these effects are present also with ARB drugs remain, however, to be studied.

5.1.2.2 Fibrinolysis

We observed no significant changes in PAI-1 activity by ramipril or doxazosin treatment. Levels of t-PA antigen tended to increase during ramipril treatment, while it was decreased by doxazosin. ACE inhibitor treatment increase the levels of bradykinin due to a reduced degradation (282), which entails anti-ischemic and antihypertensive effects due to vasodilatation, and also increased fibrinolysis (283). The tendency to increased t-PA antigen concentrations by ramipril might be due to increased levels of bradykinin. Hypertension has been associated with hypofibrinolysis with elevated of PAI-1 activity and decreased t-PA activity (284-286), and PAI-1 is related with insulin resistance, glucose intolerance, hyperinsulinemia and dyslipidemia (287). The alpha 1-adrenoceptor blocker doxazosin has been shown to improve insulin resistance, glucose intolerance, hyperinsulinemia and to improve the lipid profile (288, 289), features often present in

hypertensive patients (290). Therefore alpha 1-adrenoceptor blockers have been associated with positive effects on the fibrinolytic system, and it has been suggested that alpha 1-adrenoceptor blockers preferentially should be used in the treatment of hypertensive patients with insulin resistance (291). Our observation with a decrease in t-PA antigen and a tendency to decreased PAI-1 activity in the doxazosin treated group would implicate beneficial effects by treatment with doxazosin in hypertensive patients regarding fibrinolysis. This may be of benefit in the treatment of patients with hypofibrinolysis, such as patients with FCHL.

5.1.2.3 Inflammation

We measured several markers of inflammation (hsIL-6, IL-6R, IL-8, TNF-α, MCP-1, and hsCRP). Antihypertensive treatment overall did not affect hsIL-6, although there were directional differences between ramipril (increase) and doxazosin (decrease); and IL-6R did note change in either group. The pleiotropic cytokine IL-6 exerts proinflammatory and anti-inflammatory properties and is produced by various cells in the cardiovascular system (292). IL-6 stimulates signalling pathways in the cell when it binds to its receptor, membrane-bound IL-6 R, named classic-signalling. Signal-transduction is mediated when the IL-6/membrane-bound IL-6R complex associates with the membrane-bound receptor gp130, which dimerizes and then initiates intracellular signalling pathways. Membrane-bound IL-6R is present in few cells in the human body, especially in some leukocytes and hepatocytes. In the other pathway, named trans-signalling, circulating IL-6 binds to a soluble form of IL-6R, and this complex of IL-6/soluble IL-6R can activate most cells as gp130 is expressed uniformly in the human body. The soluble form of gp130 in human blood is considered a natural inhibitor of IL-6 trans-signalling, when binding to circulating IL-6/soluble IL-6R. The proinflammatory influences of IL-6 are associated with trans-signalling, whereas regenerative signalling and anti-inflammatory effects have been attributed to IL-6 classic-signalling (293). The concentration of soluble IL-6R and soluble gp130 in human serum is normally 1000-fold compared to IL-6, and if the increase in IL-6 is modest, the cytokine will consequently be neutralized (294). In our study the change in IL-6 was modest, while soluble IL-6R did not change. Thus, our observed increase in IL-6 in the ramipril treated group might suggest an increased classic-signalling (i.e. anti-inflammatory) cytokine action. However, IL-6 most probably acts in a paracrine fashion, and IL-6 might reach much higher concentrations in the local inflammatory areas than what we are able to measure in peripheral veins.

IL-8 is a chemotactic cytokine released by macrophages and ECs, attracting neutrophils and monocytes to the site of inflamed ECs(295). IL-8 is related with an increased incidence of future CHD (296). In our study we did not see any difference in IL-8 levels due to treatment with ramipril or doxazosin. TNF-α is a pleiotropic cytokine primarily released by monocytes and macrophages, essential in the initial activation of vascular inflammation, and is like IL-6 central in the acute phase reaction. TNF-α plays a fundamental role in endothelium dependent vasodilatation (297) and in insulin resistance (298), and is elevated

in obese subjects (299). Ang II may be one of the factors that regulate TNF-α (300). We did not observe any changes in TNF-α during ramipril or doxazosin treatment. MCP-1 is a potent chemotactic factor that regulates migration and infiltration of leukocytes across the vascular endothelium, and MCP-1 has been associated with early development of atherosclerosis (301). MCP-1 is increased by oxidative stress and by stimulating factors such as cytokines and growth factors, and the major source of MCP-1 is monocyte and macrophages (302). Ang II can induce oxidative stress and leukocyte adhesion to human ECs (303, 304). Similar to our findings with TNF-α, we observed no changes in MCP-1 levels during treatment with ramipril or doxazosin. Others have found decreased levels of MCP-1 during ACE inhibitor treatment in hypertension and in CHD (278, 305), but little has been reported on the effect of doxazosin treatment. One study has reported inhibition by doxazosin on MCP-1 directed migration of human monocytes (306). CRP is an acute-phase protein of hepatic origin, regulated by cytokines, mostly by IL-6 and TNF-α (32). In consort with the minor effects in IL-6 and TNF-α we observed no changes in hsCRP by ramipril or doxazosin. This is in line with findings in studies in hypertension with ACE inhibitors or angiotensin receptor blockers (160, 307), while doxazosin has been reported to decrease CRP, increase nitric oxide, and to decrease oxidative stress in hypertensive patients (308, 309).

In conclusion, circulating IL-6 may not be a sensitive marker to assess the potential influence of RAAS blockade on paracrine effect of IL-6 trans-signalling effects at inflammatory sites in human vessels in the clinical setting. Our results with IL-8, TNF-α, MCP-1, and hsCRP suggest that otherwise healthy patients with mild-to-moderate hypertension represent a population with a low activity of systemic inflammation, and detectable effects of antihypertensive treatment may require more advanced atherosclerotic disease.

5.2 STUDIES IN FAMILIAR HYPERLIPIDAEMIA

5.2.1 Papers III and IV

5.2.1.1 Characteristics of FCHL

FCHL exhibited higher IL-6, CRP and leukocyte counts compared to controls, whereas TNF-α level were similar in both groups. Hyperinsulinemia may influence the release of IL-6 from adipocytes, and the inflammatory mediators IL-6, CRP, fibrinogen and leucocyte count are all associated to the metabolic syndrome, without dependence of obesity (32, 310, 311). Others have calculated insulin resistance by HOMA-IR in FCHL and confirmed that patients with FCHL are associated with insulin resistance (167, 312, 313). In visceral obesity, adipocyte tissue secretes several inflammatory adipokines, such as CRP, IL-6, fibrinogen and angiotensinogen. This indicates the importance of adipocytes in inflammatory states, contributing to vasculopathy and cardiovascular risk (314). IL-6, CRP and leukocyte count are all associated with increased incidence of CVD (35-37, 315). CRP has by itself proatherogenic and proinflammatory properties and CRP may directly

induce activation of ECs (316), and increased levels of CRP is closely associated with CHD risk factors, especially obesity and deposition of visceral distribution, in individuals with or without CHD (317). Hypertriglyceridemia, but not hypercholesterolemia is associated with increased leukocyte count (318). The FCHL patients had indeed very high concentrations of triglycerides (7.4 ± 5.7 mmol/L), which possibly has an impact of our results, as triglyceride levels above 1.7 mmol/L have been reported as an independent risk factor for CHD (319). Thus, our present findings prove additional evidence for a low-grade chronic inflammation in FCHL, and also suggest that patients with FCHL, to some extent, suffer from the metabolic syndrome

The patients with FCHL exhibited an almost 6-fold higher PAI-1 activity and a 3-fold higher t-PA/PAI-1 complex level, when compared to control subjects, in agreement with previously reported studies and by us and others (27, 167, 313). PAP complex is a measure of the generation of plasmin and a marker for in vivo fibrinolysis, and increasing concentrations probably reflects increased t-PA activity (320). We observed higher PAP complex concentrations in FCHL compared to controls, which suggest that FCHL exhibited increased activities in both the activating and inhibitory proteins.

F1+2 is a in vivo thrombin generation marker, and may predict CHD (321). In our study patients with FCHL and controls had similar concentrations of F1+2 and TAT complexes, i.e. no evidence of increased thrombin generation in vivo. This is somewhat unexpected since FCHL showed a low-grade inflammatory state, and inflammation may induce coagulation through several mechanisms (induced ED, activation of platelets and the coagulation cascade through TF, suppression of anticoagulant pathways and impaired fibrinolytic activity), and others have reported that FCHL is characterized by impaired endothelium reactivity and hypercoagulability (167). One explanation could be that the FCHL participants in that study (167) had a high prevalence of CHD, which in most studies is associated with high levels of F1+2 and TAT complex (322). In contrast, our patients were otherwise healthy, not obese, and were relatively young.

Thus, patients with FCHL exhibited a low-grade chronic inflammation, and signs of impaired fibrinolysis. They also suffered from insulin resistance and showed signs of the metabolic syndrome.

5.2.1.2 Blood pressure effect during Ang II infusion

The systolic BP response to Ang II was increased in FCHL, as compared to controls, indicating that FCHL exhibited increased vascular reactivity. This is possibly a sign of ED in patients with FCHL.

5.2.1.3 Coagulation during Ang II infusion

In **paper III**, F1+2 and TAT complex values remained unchanged in FCHL and controls during infusion of Ang II. This is in contrast to a previously reported increase in TAT complex and a tendency to increased F1+2 during short-time Ang II infusion in healthy

males (13). However F1+2 exhibits a diurnal decrease during morning hours (323, 324) but the possible effects of diurnal variations of these markers were not taken into account in **paper III**. Our post hoc analyses show that TAT complex actually increased in a similar way in controls and FCHL during the *ongoing* Ang II infusion (table 17 and figure 19).This is likely to be due to the short half-life of Ang II (about 15 seconds) (325), and TAT complex (5-15 minutes) (326). When the Ang II infusion ceased, the short half-life of TAT complex resulted in a decrease in TAT levels 1 h after the end of the infusion. F1+2 has a longer half-life (90 minutes) (326), which means that the cessation of Ang II infusion did not markedly affect the concentration of F1+2 60 minutes later. We did not perform placebo infusion experiments in this study, but others have shown that TAT complex has no diurnal variation during daytime (327). Taken together, circulating Ang II seems to *increase* thrombin generation. The cause of the increased thrombin generation is unclear. Inflammatory stimuli can through several mechanisms prime the coagulation system, and a proposed mechanism is cytokine caused TF expression by the cytokines IL-6 (201). During Ang II the levels of IL-6 increased. Possibly Ang II may stimulate activation of TF, at least in part due to increased concentrations of IL-6. Platelet activation by circulating Ang II (13) may have an important contributing role in the generation of thrombin.

5.2.1.4 Fibrinolysis during Ang II infusion

In **paper III** we observed that t-PA/PAI-1 complex levels decreased in the same way in FCHL and controls during infusion of Ang II, indicating an increase in fibrinolytic activity. This suggests that Ang II stimulates fibrinolysis, rather than attenuating it, in accordance with previous results in healthy volunteers (227). Others have reported that inflammatory stimulation caused a rapid t-PA release from storage sites in ECs in chimpanzees (328). In contrast, increasing doses of Ang II, during 45 min at 3 different dose levels in subjects with normal BP, and a steady dose during 45 minutes in hypertensive subjects, resulted in increased PAI-1 antigen, while the levels of t-PA antigen remained unchanged (225). However, others have reported that increasing doses with Ang II during 45 min at 3 different dose levels affected neither PAI-1 antigen nor activity levels in normotensive, otherwise healthy subjects (226). A possible explanation for our results may be that t-PA antigen/activity and PAI-1 antigen/activity do not equal changes in t-PA/PAI-1 complexes. The doses and duration of Ang II infusions were not the same, which probably caused different hemodynamic responses. Diurnal variations of PAI-1 and t-PA during the stimulation by Ang II may also have impacted on the results (219). Finally, the increase in PAI-1 antigen seen in one of the studies (225) might have been due to an Ang II induced stimulation of t-PA activity. To note is that t-PA antigen has a diurnal decrease during morning (329), and this decrease was absent in that study, which further suggests increased t-PA levels during Ang II.

In **paper IV** PAI-1 activity decreased similarly in healthy controls and FH during Ang II infusion, which most likely reflects a diurnal decrease in PAI-1 activity (330). Others have reported falling levels of PAI-1 activity during morning hours (219), which is confirmed by

our placebo infusion study. Ang II has been shown to stimulate cultured human coronary ECs in a time dependent manner, to release PAI-1 protein and even very low levels of Ang II induced PAI-1 protein, which could be detected already after 6 h (331). Others have reported that Ang II increased PAI-1 messenger RNA expression in cultured human adipocytes after 3 h and PAI-1 protein release after 12 h (332). Hence, it has been argued that increased levels of Ang II are closely associated with elevated levels of PAI-1. On the contrary, studies by us and others show that PAI-1, is unaffected by Ang II infusion (226, 227). Ang II may induce PAI-1 release in the *long-term* setting, but we could not find any *short-term* impact of Ang II on the concentrations of PAI-1.

PAP complex concentrations remained unchanged during infusion of Ang II in FCHL, while these levels increased in controls. PAP complex levels remained unchanged with placebo infusion, suggesting no diurnal variation in PAP complex during morning hours. It has also been reported that the major plasmin inhibitor alpha-2 antiplasmin lacks diurnal variation (330). As PAP complex is a measure of the generation of plasmin and a marker for in vivo fibrinolysis, we propose that the increased PAP complex levels seen in the controls during stimulation by Ang II us caused by an increase in t-PA activity, in accordance with previous findings in vitro and in healthy volunteers (227, 331). The "non-response" of PAP complex concentrations during infusion of Ang II in FCHL, reflecting unchanged t-PA activity, may suggest incapacity of the ECs to release t-PA in response to Ang II, compatible with ED in FCHL. Taken together, Ang II seems to increase the fibrinolytic capacity by an increased t-PA activity concentration, at least in the short run, and argue against the claim that a brief stimulation by Ang II impairs fibrinolysis. Contrary to previous claims, we could not observe any short-term impact on PAI-1 activity by Ang II stimulation.

5.2.1.5 Inflammation during Ang II infusion

Ang II caused a 2- to 3-fold increase in IL-6, similar in FCHL and in healthy controls. This is in accordance with findings in rat VSMCs in vitro and in healthy controls showing a dose dependent release of IL-6 (106, 333). IL-6 may be produced by a number of cells and the origin of IL-6 by Ang II stimulation cannot be determined from our study. Unfortunately, we did not measure soluble IL-6R, and therefore it is doubtful to estimate the proinflammatory or anti-inflammatory effects due to the increase of IL-6 concentrations. The possible paracrine increase of IL-6 levels at inflammatory sites is generally difficult to measure. The marked increase in IL-6 in our study could suggest that IL-6 may have reached even higher levels at local areas in vessels, which may represent a local increase of trans-signalling, and a proinflammatory effect. Due to the substantial increase in IL-6 levels during Ang II infusion we suggest that Ang II may have proinflammatory effects.

The leukocyte count increased in a similar way in FCHL and controls during infusion of Ang II, and this is in line with results reported by others (334). The mechanism for this

increase is unknown, but the Ang II stimulated response in leukocyte count supports the assumption that Ang II may exert proinflammatory effects.

TNF-α concentration remained unchanged by the infusion of Ang II, and this extends observations reported by others (335). Probably stimulation during longer periods of time by Ang II may be necessary to stimulate TNF-α synthesis and release, as indicated by animal experiments (336). In any case we can conclude that TNF-α was not the cause for the observed increase in IL-6 in our study.

5.2.2 Paper V

5.2.2.1 Characteristics of FH

IL-6, leukocyte count and CRP concentrations remained similar in FH and controls, suggesting no convincing signs of systemic inflammation in FH.

FH and controls had comparable levels of PAI-1 activity. Furthermore, PAP complex levels were equal in FH and control subjects. Thus, FH patients exhibit an intact and unaffected fibrinolytic system.

An association between F1+2 levels and total cholesterol and LDL cholesterol levels has been reported in hypercholesterolemic patients in advance of atherosclerotic CVD events (337). In our study the F1+2 concentrations were not different in FH patients and in controls. However using the CAT method, the thrombin generation potential was higher in FH compared to controls, in accordance with the assumption that FH is a prothrombotic state. We observed higher fibrinogen concentrations in FH compared to controls, in agreement with others (338, 339). As data has been presented indicating that fibrinogen mediates inflammation beyond its central importance in the coagulation cascade, we speculate that the observed high fibrinogen levels in FH subjects imply activation of the coagulation system and represent signs of early, silent atherosclerosis.

In conclusion, FH showed an intact fibrinolysis, while the thrombin generation potential, i.e. significantly higher levels of the CAT parameters peak thrombin concentration and endogenous thrombin potential, was increased compared to controls.

5.2.2.2 Blood pressure effects during Ang II infusion

FH did not show any signs of increased vascular responsiveness to Ang II.

5.2.2.3 Coagulation during Ang II infusion

F1+2 levels remained unchanged in FH and controls during Ang II infusion, in spite of the elevated IL-6 levels. The absence of the expected diurnal decrease in F1+2 concentrations during morning hours, during stimulation by Ang II, may be considered as a relative increase in thrombin generation, similar to findings in healthy subjects (13) and in FCHL patients (340). Regarding CAT analyses, Ang II did not affect the concentration dependent thrombin generation data, peak thrombin concentrations and endogenous thrombin

potential. Instead we observed an effect on the time dependent parameters in FH patients, i.e. slightly prolonged lag-time, time to peak thrombin concentration, and time to tail. How to interpret these results is somewhat unclear. Increased time to peak thrombin has been reported in acute coronary syndromes (341, 342), possibly due to increased TFPI plasma levels (341). One may speculate that the putative endothelial perturbation in patients with FH may cause EC release of TFPI during infusion of Ang II, which binds to TF added in the CAT analysis and leads to the delay of thrombin generation. This might be taken to suggest that patients with FH exhibit an activation of the anticoagulant system. We conclude that Ang II affects thrombin generation in the short run. The increase in fibrinogen concentrations were modest during infusion of Ang II, while the most important regulator of fibrinogen, IL-6, increased. We did not measure changes in fibrinogen during placebo infusion, but others have shown that fibrinogen does not have a diurnal variation during morning hours (329). Others have reported that fibrinogen responds to stimulation by IL-6 with a long delay of time (266). Thus, the duration of our experiments was probably too short to cause a substantial change in fibrinogen levels.

5.2.2.4 Fibrinolysis during Ang II infusion

PAI-1 activity decreased during infusion of Ang II, similarly in FH and controls. A similar decrease occurred during placebo infusion. Our results thus reflect the diurnal variation in PAI-1 activity (330). PAP complex increased similarly in FH and controls during Ang II. As alpha-2 antiplasmin lacks diurnal variation (330), and PAP complex did not show any diurnal variation in our placebo infusion group, we conclude that the increase in PAP complex levels were caused by an increase in t-PA activity, in accordance with our previous results (227). Thus, Ang II causes an acute increase in fibrinolysis.

5.2.2.5 Inflammation during Ang II infusion

Ang II stimulation caused a marked increase in both IL-6 and leukocyte count, similarly in both groups, extending reports in healthy subjects (13, 333, 334) and in accordance with our finding in FCHL and healthy control subjects. Although the origin of IL-6 or leukocyte count cannot be determined, our results indicate that Ang II may have proinflammatory properties.

Thus, we suggest that patients with FH have an increased thrombin generation, rather than alterations in the fibrinolytic activity or in inflammation, and this contributes to the increased risk of future CHD and premature death in FH. We also suggest that Ang II in FH, in the short run, acts as a proinflammatory, profibrinolytic and a prothrombotic agent.

6 CONCLUSIONS

- Antihypertensive treatment with the ACE inhibitor ramipril reduces thrombin generation and tends to lowers fibrinogen concentrations. This effect of ramipril on thrombin generation goes beyond the effects on BP reduction alone. Thus, drugs blocking the renin-angiotensin-aldosterone system may reduce atherothrombotic complications beyond their effects to reduce BP.

- Our observation that doxazosin decreased t-PA antigen and a tended to decrease PAI-1 activity would implicate beneficial effects by treatment with doxazosin in hypertensive patients regarding fibrinolysis. This may be of value in the treatment of patients with hypofibrinolysis, such as patients with FCHL.

- Patients with FCHL have signs of an ongoing low-grade systemic inflammation and impaired fibrinolysis, while the coagulation system is intact. In contrast, patients with FH have an activated coagulation, intact fibrinolysis but no convicing signs of systemic inflammation.

- In contrast to FH, patients with FCHL share several charateristics with the metabolic syndrome, including high triglyceride and low HDL cholesterol levels, insulin resistance and high body mass index.

- In healthy individuals circulating Ang II acts as a proinflammatory agent and may increase thrombin generation and fibrinolysis. Since inflammation participates in vascular remodelling and atherosclerosis, Ang II may contribute to vascular dysfunction in hypertension.

- In patients with FCHL and FH, Ang II acts as a proinflammatory agent and may increase thrombin generation. Patients with FCHL appear to be incapable of increasing fibrinolysis in response to circulating Ang II, while patients with FH, on the contrary, seem to increase their fibrinolysis.

- In contrast to previous claims, we observed no effects of short term circulating Ang II on PAI-1 activity in healthy individuals or in patients with familial hyperlipidaemia.

- Thus, blocking the renin-angiotensin-aldosterone system by an ACE inhibitor may prevent atherothrombotic complications in hypertensive patients by reducing thrombin formation beyond the effects on BP. Different mechanisms may contribute to the increased incidence of cardiovascular complications in patients with FCHL and FH. ACE inhibitor treatment might be of benefit in patients with FCHLby reducing inflammation and insulin resistance, and in patients with FH mainly by reducing thrombin generation.

7 FUTURE PERSPECTIVES

Treatment with the ACE inhibitor ramipril reduced thrombin generation beyond the effects on BP reduction alone (**Paper II**). Possibly the reduced levels of TAT complex reflect an attenuated thrombin generation which is, at least partly, due to a reduced expression of tissue factor on MPs originating from ECs and/or blood cells To get a better understanding of such mechanisms, one should measure plasma concentrations of MPs exposing tissue factor, as well as its origins.

FH showed an intact fibrinolysis while the thrombin generation potential, i.e. significantly higher levels of the CAT parameters peak thrombin concentration and endogenous thrombin potential were increased, as compared to controls **(paper V)**. Possibly the increased thrombin generation, measured by TAT complex, in FH may be due to an increased expression of tissue factor on MPs originating from ECs and/or blood cells. To study the mechanisms involved, it would be worthwhile to examine plasma concentrations of MPs exposing tissue factor, as well as its origins.

ARB also inhibits RAAS, but through a different mechanism compared to ACE inhibitors. Blocking AT1R with an ARB will inhibit the negative feed-back on renin with increased renin concentrations and subsequently an increase in Ang II, while bradykinin levels are unaffected. Thus, ARB treatment may activate AT2R, which mediates effects generally opposite to the effects mediated by AT1R. Hence, several different mechanisms are in action when using an ARB, compared to the use of an ACE inhibitor. Therefore, it would be interesting to study the effects on inflammation and haemostasis in hypertensive patients and to compare treatment of an ARB to treatment with doxazosin, i.e. a similar design as in paper II, but changing the ACE inhibitor to an ARB.

Oxidized LDL induces TF expression in ECs and monocytes, and patients with raised LDL cholesterol levels exhibit increased TF plasma activity. Statins have been shown to reduce TF expression in ECs, monocytes and VSMCs. It would be interesting to examine the origin of TF expression in combination therapy with an ACE inhibitor and a statin and to compare with statin treatment alone in patients with high concentrations of cholesterol, such as FH.

8 SVENSK SAMMANFATTNING

Åderförkalkning är multifaktoriell och endoteldysfunktion (begynnande funktionsskada i kärlväggen) anses föregå åderförkalkning. Angiotensin (Ang) II, den huvudsakliga effektorn av renin-angiotensin-aldosteronsystemet (RAAS), är inblandad i hypertoni och har visat sig bidra till åderförkalkning. Läkemedlet ”angiotensin converting enzyme” (ACE) hämmare minskar bildningen av Ang II.

Två vanliga blodfettsrubbningar; familjär kombinerad hyperlipidemi (FCHL) och familjär hyperkolesterolemi (FH), har båda identifierats som riskfaktorer för hjärt-kärlsjukdomar och för tidig död. FCHL har en annan fenotyp (observerbara egenskaper) jämfört med FH, men båda associeras med endoteldysfunktion och en subklinisk åderförkalkning. Vi spekulerade i att patienter med hypertoni samt blodfettsrubbningarna FCHL och FH var mer känsliga för de potentiella proinflammatoriska och prokoagulatoriska effekterna av Ang II jämfört med friska individer. Vi undersökte därför hur RAAS påverkar inflammation och koagulation hos friska kontroller samt hos individer med hypertoni och FCHL respektive FH.

I **delarbete I** undersökt vi effekten av behandling med ACE hämmaren ramipril avseende koagulation hos patienter med mild-till-måttlig hypertoni. Vi observerade att ramipril dämpade bildningen av trombin (förorsakar blodkoagulering) hos individer med essentiell (utan känd orsak) hypertoni. Vi såg också en tendens till minskade nivåer av fibrinogen.

I **delarbete II** ville vi klargöra effekten av blodtryckssänkande behandling i sig. Därför undersökt vi effekterna av 3 månaders behandling med ramipril jämfört med doxazosin, som är en blodtrycksmedicin utan någon påverkan på RAAS, avseende inflammation och koagulation hos patienter med mild-till-måttlig hypertoni. Vi fann att blodtryckssänkande behandling i sig hade en begränsad effekt på systemisk inflammation. Behandling med ramipril, men inte doxazosin, tycktes minska bildningen av trombin. Detta utökade våra tidigare resultat från **delarbete I** genom att vi här visade att behandling med ramipril minskade trombin bildningen, utöver de blodtryckssänkande effekterna. Läkemedel som blockerar RAAS kan därmed minska aterotrombotiska komplikationer utöver de blodtryckssänkande effekterna. Vi observerade också fördelaktiga effekter på fibrinolysen (system som löser upp blodkoagler), som skulle unna tala för positiva effekter av behandling med doxazosin hos hypertensiva patienter avseende fibrinolys. Detta kan vara till nytta vid behandling av patienter med nedsatt fibrinolys, såsom patienter med FCHL.

I **delarbete III** undersökte vi hur en intravenös infusion av Ang II under 3 timmar påverkade inflammation och koagulation hos patienter med FCHL och friska kontrollpersoner. I **Delarbete IV** beskrev vi patienterna med FCHL i **delarbete III**, ytterligare med avseende på deras insulinresistens och fibrinolys. Vi genomförde också placebo experiment för att göra det möjligt att bedöma påverkan av de dagliga variationerna och kontrollera stabiliteten i vår experimentella design. Vi fann att FCHL hade ett förhöjt systoliskt blodtryckssvar under infusionen av Ang II jämfört med

kontroller, vilket skulle kunna tala för en ökad endoteldysfunktion hos FCHL. Patienter med FCHL uppvisade en låggradig kronisk inflammation och en nedsatt fibrinolys, medan koagulationssystemet däremot föreföll vara intakt. FCHL hade flera kännetecken för det metabola syndromet; höga triglycerid och låga HDL kolesterol nivåer, insulinresistens och ett högt kroppsmasseindex. Infusion av Ang II ökade systemisk inflammation på ett liknande sätt hos FCHL och kontroller. Ang II medförde inte någon påverkan på bildandet av trombin, vare sig hos FCHL eller hos kontrollerna. Ang II påverkade inte fibrinolysen hos FCHL, medan fibrinolysen däremot ökade hos de friska kontrollerna. De olika svaren för Ang II stimuleringen berodde förmodligen på att patienter med FCHL är oförmögna att öka sin fibrinolys som svar på Ang II. Vi kunde inte observera några kortsiktiga effekter på PAI-1-aktiviteten, varken hos FCHL eller hos kontrollerna. Våra fynd talar för att patienter med FCHL har en låggradig kronisk inflammation, en nedsatt fibrinolytisk kapacitet och flera kännetecken för det metabola syndromet, som alla bidrar till den ökade risken för hjärt-kärlsjukdomar och för tidig död hos FCHL. Vi föreslog också att Ang II verkar proinflammatoriskt och förorsakar en ökad fibrinolys, utan någon inverkan på trombin bildningen. Effekterna av de dagliga variationerna av våra markörer för koagulationen togs inte i beaktande i **delarbete III**. Om effekterna av Ang II däremot analyseras under den *faktiska* infusionstiden, visade nya data istället att bildandet av trombin istället ökade hos både FCHL och kontrollerna. Vår nya slutsats blev därför istället att Ang II verkar protrombotiskt.

I **delarbete V** undersökte vi hur en intravenös infusion av Ang II under 3 timmar påverkade inflammation och koagulation hos patienter med FH och friska kontroller. Vi genomförde också placebo experiment för att göra det möjligt att bedöma påverkan av de dagliga variationerna och kontrollera stabiliteten i vår experimentella design. Vi fann att patienter med FH hade högre systoliskt blodtryck än kontrollerna, medan blodtryckssvaren var lika hos FH och kontrollerna. FH uppvisade en intakt fibrinolys, men en ökad potential att bilda trombin jämfört med kontrollerna. FH hade inte några övertygande tecken på en pågående låggradig inflammation. Infusion av Ang II orsakade en ökning i inflammation, fibrinolys och troligen bildandet av trombin, på samma sätt hos både FH och kontrollerna. Under Ang II infusionen visade FH tecken på ett aktiverat antikoagulerande system. Vi föreslog att patienter med FH har ett aktiverat koagulationssystem i stället för en förändrad fibrinolys eller inflammation, vilket kan bidra till den ökade risken för hjärt-kärlsjukdomar och för tidig död hos FH.

Vår slutsats blev att blockering av RAAS genom att använda en ACE hämmare kan förhindra aterotrombotiska komplikationer hos patienter med hypertoni, utöver effekterna av blodtryckssänkningen framför allt genom att minska bildandet av trombin. Olika mekanismer kan bidra till ökningen av kardiovaskulära komplikationer hos patienter med FCHL och FH. Hos patienter med FCHL kan en effekt av ACE hämning vara att dämpa inflammation, i kombination med deras kända positiva inverkan på insulinresistens, och hos patienter med FH förefaller den huvudsakliga effekten vara att minska bildningen av trombin.

9 ACKNOWLEDGMENTS

This research program was conducted at the Clinical Research Centre (Cardiovascular Research Laboratory, Microcirculation and Metabolic Laboratory, and Biochemistry Research Laboratory) at Danderyd University Hospital and Karolinska Institutet, Department of Clinical Sciences, Danderyd Hospital. I wish to express my sincere gratitude to all who have supported me and contributed to this thesis, and in particular I would like to mention:

Professor **Thomas Kahan**, supervisor, for introducing me to the field of research and assisting me throughout the articles and this thesis, for finding time to read my manuscripts and giving quick feedback. Thank you for your enthusiasm and for believing in me. Not least, thank you for pushing me!

Professor **Håkan Wallén**, assistant supervisor**,** for expertise in haemostasis and highly skilled knowledge in research. You have a lot of commitments, but despite that set of time for my project, your advices have been invaluable.

Andreas Jekell, MD, PhD student, and co-author of **paper II,** for designing and inclusion of the patients in **paper II,** and for valuable comments on the manuscript.

Associate Professor **Gun Jörneskog**, co-author of **paper III-V**, for all the support and help during the experiments at the Microcirculation and Metabolic Laboratory.

Associate Professor **Anders Bröijersén**, co-author, for the recruitment of patients with familial combined hyperlipidaemia from the Centre for Metabolism and Endocrinology at the Karolinska University Hospital, Huddinge, which made **papers III** and **IV** come true.

Associate Professor **Jonas Brinck**, co-author, for the recruitment of patients with familial hypercholesterolemia from the Centre for Metabolism and Endocrinology at the Karolinska University Hospital, Huddinge, which made **paper V** come true.

Associate Professor **Keith Eliasson**, co-author, for valuable contribution to **paper I.**

Associate professor **Hans Jonsson**, co-author, for valuable contribution to **paper I.**

Associate Professor **Bruna Gigante,** co-author, for your help and skill in designing the how to and what to analyse in **paper II,** and for the opportunity to send all blood samples to Karolinska Institutet, Institute of Environmental Medicine for analyses.

Professor **Margareta Blombäck**, for providing analyses of coagulation parameters and for valuable comments to **paper I.**

Karin Malmqvist, MD, PhD, head of Cardiology at Danderyd University Hospital; the best boss I have ever had, for your support when I was in charge of unit 97, for creating research conditions for me during the initial phase of this research program.

Richard Müller-Brunotte, MD, PhD, former colleague and dear friend, for endless discussions of life in the student city of Uppsala and for sharing my interest in golf.

Associate professor **Jonas Spaak,** for saving me in the last minute, you know why!

Ann-Christine Salomonsson, for expert technical assistance, kindness and nice companionship during our experiments, and endless thank you for the structure and order of our patient data.

Camilla Andersson and **Madeleine Derewand**, for expert technical assistance and nice companionship during our experiments. We spent a lot of hours in the laboratory during all our experiments with Ang II infusions.

Graciela Elgue, for expert technical assistance and nice companionship during all our experiments and analyses. Especially the PAP analyses were dependent on your expertise.

Katherina Aguilera Gatica, for expert technical assistance and nice companionship during our experiments and analyses. You really helped me a lot with the analyses in **papers IV** and **V,** and always got back to me with quick answers to my e-mails.

Ingemar Steinbruck, for expert technical assistance and nice companionship during all our experiments in the old laboratory down at Edsviken.

Parisa Baniasad, for expert technical assistance and nice companionship during the experiments.

My wife **Maria** and my children **Melker**, **Carl-Johan** and **Gustaf,** for love and support, and for giving me the opportunity to spend time doing my research and enjoying all aspects of life outside the walls of the hospital.

My mother **Vivi-Ann**, for being such a good mother, for your never-ending energy and for always encouraging me in what I have done. You have always supported me in the best of ways.

My father **Karl-Evert**, for love and support and for providing the necessary platform for life and education. Unfortunately you did not get the opportunity to see the final result of my research project.

All my friends at the Department of Cardiology, Danderyds Hospital, Stockholm, Sweden.

All my friends at the Department of Internal Medicine, Division of Cardiovascular Medicine, Ryhov County Hospital, Jönköping, Sweden.

And last but not least; to all **study participants** who made this thesis possible. Your willingness to engage in clinical research is admirable and of fundamental importance.

The studies were supported by grants from Futurum, the academy of Health Care, Jönköping County Council; the Swedish Heart-Lung Foundation; Karolinska Institutet Research Foundations; the Swedish Society of Medicine; AstraZeneca AB (Sweden); and an unrestricted grant from Pfizer Inc.

10 REFERENCES

1. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med. 2006;3(11):e442.
2. Leal J, Luengo-Fernandez R, Gray A, Petersen S, Rayner M. Economic burden of cardiovascular diseases in the enlarged European Union. Eur Heart J. 2006;27(13):1610-9.
3. Kotseva K, Wood D, De Backer G, De Bacquer D, Pyorala K, Keil U. EUROASPIRE III: a survey on the lifestyle, risk factors and use of cardioprotective drug therapies in coronary patients from 22 European countries. Eur J Cardiovasc Prev Rehabil. 2009;16(2):121-37.
4. Volpe M. Should all patients at high cardiovascular risk receive renin-angiotensin system blockers? Qjm. 2012;105(1):11-27.
5. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004;364(9438):937-52.
6. O'Neill S, O'Driscoll L. Metabolic syndrome: a closer look at the growing epidemic and its associated pathologies. Obes Rev. 2015;16(1):1-12.
7. Weiss D, Kools JJ, Taylor WR. Angiotensin II-induced hypertension accelerates the development of atherosclerosis in apoE-deficient mice. Circulation. 2001;103(3):448-54.
8. Hernandez-Presa M, Bustos C, Ortego M, Tunon J, Renedo G, Ruiz-Ortega M, et al. Angiotensin-converting enzyme inhibition prevents arterial nuclear factor-kappa B activation, monocyte chemoattractant protein-1 expression, and macrophage infiltration in a rabbit model of early accelerated atherosclerosis. Circulation. 1997;95(6):1532-41.
9. Heart Outcomes Prevention Evaluation Study Investigators. Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICRO-HOPE substudy. Heart Outcomes Prevention Evaluation Study Investigators. Lancet. 2000;355(9200):253-9.
10. Nishimura H, Tsuji H, Masuda H, Nakagawa K, Nakahara Y, Kitamura H, et al. Angiotensin II increases plasminogen activator inhibitor-1 and tissue factor mRNA expression without changing that of tissue type plasminogen activator or tissue factor pathway inhibitor in cultured rat aortic endothelial cells. Thromb Haemost. 1997;77(6):1189-95.
11. He M, He X, Xie Q, Chen F, He S. Angiotensin II induces the expression of tissue factor and its mechanism in human monocytes. Thromb Res. 2006;117(5):579-90.
12. Napoleone E, Di Santo A, Camera M, Tremoli E, Lorenzet R. Angiotensin-converting enzyme inhibitors downregulate tissue factor synthesis in monocytes. Circ Res. 2000;86(2):139-43.
13. Larsson PT, Schwieler JH, Wallen NH. Platelet activation during angiotensin II infusion in healthy volunteers. Blood Coagul Fibrinolysis. 2000;11(1):61-9.

14. Daugherty A, Lu H, Rateri DL, Cassis LA. Augmentation of the renin-angiotensin system by hypercholesterolemia promotes vascular diseases. Future Lipidol. 2008;3(6):625-36.

15. Daugherty A, Manning MW, Cassis LA. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. J Clin Invest. 2000;105(11):1605-12.

16. Hayek T, Attias J, Coleman R, Brodsky S, Smith J, Breslow JL, et al. The angiotensin-converting enzyme inhibitor, fosinopril, and the angiotensin II receptor antagonist, losartan, inhibit LDL oxidation and attenuate atherosclerosis independent of lowering blood pressure in apolipoprotein E deficient mice. Cardiovasc Res. 1999;44(3):579-87.

17. Chobanian AV, Haudenschild CC, Nickerson C, Hope S. Trandolapril inhibits atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. Hypertension. 1992;20(4):473-7.

18. Hayek T, Attias J, Smith J, Breslow JL, Keidar S. Antiatherosclerotic and antioxidative effects of captopril in apolipoprotein E-deficient mice. J Cardiovasc Pharmacol. 1998;31(4):540-4.

19. Pfeffer MA, Braunwald E, Moye LA, Basta L, Brown EJ, Jr., Cuddy TE, et al. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. Results of the survival and ventricular enlargement trial. The SAVE Investigators. N Engl J Med. 1992;327(10):669-77.

20. The SOLVD Investigators. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. The SOLVD Investigattors. N Engl J Med. 1992;327(10):685-91.

21. Morawietz H, Rueckschloss U, Niemann B, Duerrschmidt N, Galle J, Hakim K, et al. Angiotensin II induces LOX-1, the human endothelial receptor for oxidized low-density lipoprotein. Circulation. 1999;100(9):899-902.

22. Fredrickson DS, Lees RS. A system for phenotyping hyperlipoproteinemia. Circulation. 1965;31:321-7.

23. De Michele M, Iannuzzi A, Salvato A, Pauciullo P, Gentile M, Iannuzzo G, et al. Impaired endothelium-dependent vascular reactivity in patients with familial combined hyperlipidaemia. Heart. 2007;93(1):78-81.

24. Simpson HC, Mann JI, Meade TW, Chakrabarti R, Stirling Y, Woolf L. Hypertriglyceridaemia and hypercoagulability. Lancet. 1983;1(8328):786-90.

25. Dichtl W, Nilsson L, Goncalves I, Ares MP, Banfi C, Calara F, et al. Very low-density lipoprotein activates nuclear factor-kappaB in endothelial cells. Circ Res. 1999;84(9):1085-94.

26. van Oostrom AJ, van Wijk J, Cabezas MC. Lipaemia, inflammation and atherosclerosis: novel opportunities in the understanding and treatment of atherosclerosis. Drugs. 2004;64 Suppl 2:19-41.

27. Mussoni L, Mannucci L, Sirtori M, Camera M, Maderna P, Sironi L, et al. Hypertriglyceridemia and regulation of fibrinolytic activity. Arterioscler Thromb. 1992;12(1):19-27.

28. Sorensen KE, Celermajer DS, Georgakopoulos D, Hatcher G, Betteridge DJ, Deanfield JE. Impairment of endothelium-dependent dilation is an early event in children with familial hypercholesterolemia and is related to the lipoprotein(a) level. J Clin Invest. 1994;93(1):50-5.

29. Avellone G, Di Garbo V, Cordova R, Abruzzese G, Rotolo G, De Simone R, et al. Activation of coagulation but normal fibrinolysis in patients with type IIA hyperlipoproteinemia. Thromb Res. 1996;81(2):277-82.

30. Huijgen R, Kastelein JJ, Meijers JC. Increased coagulation factor VIII activity in patients with familial hypercholesterolemia. Blood. 2011;118(26):6990-1.

31. Kalsch T, Elmas E, Nguyen XD, Suvajac N, Kluter H, Borggrefe M, et al. Endotoxin-induced effects on platelets and monocytes in an in vivo model of inflammation. Basic Res Cardiol. 2007;102(5):460-6.

32. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol. 1999;19(4):972-8.

33. Verma S, Wang CH, Li SH, Dumont AS, Fedak PW, Badiwala MV, et al. A self-fulfilling prophecy: C-reactive protein attenuates nitric oxide production and inhibits angiogenesis. Circulation. 2002;106(8):913-9.

34. Devaraj S, Xu DY, Jialal I. C-Reactive Protein Increases Plasminogen Activator Inhibitor-1 Expression and Activity in Human Aortic Endothelial Cells: Implications for the Metabolic Syndrome and Atherothrombosis. Circulation. 2003;107(3):398-404.

35. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation. 2000;101(15):1767-72.

36. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Plasma concentration of C-reactive protein and risk of developing peripheral vascular disease. Circulation. 1998;97(5):425-8.

37. Kaptoge S, Di Angelantonio E, Pennells L, Wood AM, White IR, Gao P, et al. C-reactive protein, fibrinogen, and cardiovascular disease prediction. N Engl J Med. 2012;367(14):1310-20.

38. Di Angelantonio E, Gao P, Pennells L, Kaptoge S, Caslake M, Thompson A, et al. Lipid-related markers and cardiovascular disease prediction. Jama. 2012;307(23):2499-506.

39. Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. J Clin Invest. 2005;115(12):3378-84.

40. Cadenas E, Sies H. Oxidative stress: excited oxygen species and enzyme activity. Adv Enzyme Regul. 1985;23:217-37.

41. Dubey RK, Jackson EK, Luscher TF. Nitric oxide inhibits angiotensin II-induced migration of rat aortic smooth muscle cell. Role of cyclic-nucleotides and angiotensin1 receptors. J Clin Invest. 1995;96(1):141-9.

42. De Caterina R, Libby P, Peng HB, Thannickal VJ, Rajavashisth TB, Gimbrone MA, Jr., et al. Nitric oxide decreases cytokine-induced endothelial activation. Nitric

oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. J Clin Invest. 1995;96(1):60-8.

43. Peng HB, Libby P, Liao JK. Induction and stabilization of I kappa B alpha by nitric oxide mediates inhibition of NF-kappa B. J Biol Chem. 1995;270(23):14214-9.

44. Collins T, Read MA, Neish AS, Whitley MZ, Thanos D, Maniatis T. Transcriptional regulation of endothelial cell adhesion molecules: NF-kappa B and cytokine-inducible enhancers. Faseb j. 1995;9(10):899-909.

45. Deanfield J, Donald A, Ferri C, Giannattasio C, Halcox J, Halligan S, et al. Endothelial function and dysfunction. Part I: Methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. J Hypertens. 2005;23(1):7-17.

46. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med. 1999;340(2):115-26.

47. Massberg S, Brand K, Gruner S, Page S, Muller E, Muller I, et al. A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. J Exp Med. 2002;196(7):887-96.

48. Gawaz M. Role of platelets in coronary thrombosis and reperfusion of ischemic myocardium. Cardiovasc Res. 2004;61(3):498-511.

49. Weingart C, Nelson PJ, Kramer BK, Mack M. Dose dependent effects of platelet derived chondroitinsulfate A on the binding of CCL5 to endothelial cells. BMC Immunol. 2008;9:72.

50. Diacovo TG, Roth SJ, Buccola JM, Bainton DF, Springer TA. Neutrophil rolling, arrest, and transmigration across activated, surface-adherent platelets via sequential action of P-selectin and the beta 2-integrin CD11b/CD18. Blood. 1996;88(1):146-57.

51. Smiley ST, King JA, Hancock WW. Fibrinogen stimulates macrophage chemokine secretion through toll-like receptor 4. J Immunol. 2001;167(5):2887-94.

52. Hsu-Lin S, Berman CL, Furie BC, August D, Furie B. A platelet membrane protein expressed during platelet activation and secretion. Studies using a monoclonal antibody specific for thrombin-activated platelets. J Biol Chem. 1984;259(14):9121-6.

53. McEver RP, Beckstead JH, Moore KL, Marshall-Carlson L, Bainton DF. GMP-140, a platelet alpha-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. J Clin Invest. 1989;84(1):92-9.

54. Middleton J, Neil S, Wintle J, Clark-Lewis I, Moore H, Lam C, et al. Transcytosis and surface presentation of IL-8 by venular endothelial cells. Cell. 1997;91(3):385-95.

55. von Hundelshausen P, Weber KS, Huo Y, Proudfoot AE, Nelson PJ, Ley K, et al. RANTES deposition by platelets triggers monocyte arrest on inflamed and atherosclerotic endothelium. Circulation. 2001;103(13):1772-7.

56. Weber C, Springer TA. Neutrophil accumulation on activated, surface-adherent platelets in flow is mediated by interaction of Mac-1 with fibrinogen bound to

alphaIIbbeta3 and stimulated by platelet-activating factor. J Clin Invest. 1997;100(8):2085-93.

57. Hoogewerf AJ, Kuschert GS, Proudfoot AE, Borlat F, Clark-Lewis I, Power CA, et al. Glycosaminoglycans mediate cell surface oligomerization of chemokines. Biochemistry. 1997;36(44):13570-8.

58. Ghasemzadeh M, Hosseini E. Platelet-leukocyte crosstalk: Linking proinflammatory responses to procoagulant state. Thromb Res. 2013;131(3):191-7.

59. Zarbock A, Deem TL, Burcin TL, Ley K. Galphai2 is required for chemokine-induced neutrophil arrest. Blood. 2007;110(10):3773-9.

60. Sigal A, Bleijs DA, Grabovsky V, van Vliet SJ, Dwir O, Figdor CG, et al. The LFA-1 integrin supports rolling adhesions on ICAM-1 under physiological shear flow in a permissive cellular environment. J Immunol. 2000;165(1):442-52.

61. Putnam K, Shoemaker R, Yiannikouris F, Cassis LA. The renin-angiotensin system: a target of and contributor to dyslipidemias, altered glucose homeostasis, and hypertension of the metabolic syndrome. Am J Physiol Heart Circ Physiol. 2012;302(6):H1219-30.

62. Marcus Y, Shefer G, Stern N. Adipose tissue renin-angiotensin-aldosterone system (RAAS) and progression of insulin resistance. Mol Cell Endocrinol. 2013;378(1-2):1-14.

63. Qi Y, Li H, Shenoy V, Li Q, Wong F, Zhang L, et al. Moderate cardiac-selective overexpression of angiotensin II type 2 receptor protects cardiac functions from ischaemic injury. Exp Physiol. 2012;97(1):89-101.

64. Santos RA, Simoes e Silva AC, Maric C, Silva DM, Machado RP, de Buhr I, et al. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. Proc Natl Acad Sci U S A. 2003;100(14):8258-63.

65. Bader M. ACE2, angiotensin-(1-7), and Mas: the other side of the coin. Pflugers Arch. 2013;465(1):79-85.

66. Sampaio WO, Souza dos Santos RA, Faria-Silva R, da Mata Machado LT, Schiffrin EL, Touyz RM. Angiotensin-(1-7) through receptor Mas mediates endothelial nitric oxide synthase activation via Akt-dependent pathways. Hypertension. 2007;49(1):185-92.

67. Gwathmey TM, Pendergrass KD, Reid SD, Rose JC, Diz DI, Chappell MC. Angiotensin-(1-7)-angiotensin-converting enzyme 2 attenuates reactive oxygen species formation to angiotensin II within the cell nucleus. Hypertension. 2010;55(1):166-71.

68. Fraga-Silva RA, Savergnini SQ, Montecucco F, Nencioni A, Caffa I, Soncini D, et al. Treatment with Angiotensin-(1-7) reduces inflammation in carotid atherosclerotic plaques. Thromb Haemost. 2014;111(4):736-47.

69. Park JB, Schiffrin EL. Cardiac and vascular fibrosis and hypertrophy in aldosterone-infused rats: role of endothelin-1. Am J Hypertens. 2002;15(2 Pt 1):164-9.

70. Virdis A, Neves MF, Amiri F, Viel E, Touyz RM, Schiffrin EL. Spironolactone improves angiotensin-induced vascular changes and oxidative stress. Hypertension. 2002;40(4):504-10.

71. Mazak I, Fiebeler A, Muller DN, Park JK, Shagdarsuren E, Lindschau C, et al. Aldosterone potentiates angiotensin II-induced signaling in vascular smooth muscle cells. Circulation. 2004;109(22):2792-800.

72. Nakano S, Kobayashi N, Yoshida K, Ohno T, Matsuoka H. Cardioprotective mechanisms of spironolactone associated with the angiotensin-converting enzyme/epidermal growth factor receptor/extracellular signal-regulated kinases, NAD(P)H oxidase/lectin-like oxidized low-density lipoprotein receptor-1, and Rho-kinase pathways in aldosterone/salt-induced hypertensive rats. Hypertens Res. 2005;28(11):925-36.

73. Caprio M, Newfell BG, la Sala A, Baur W, Fabbri A, Rosano G, et al. Functional mineralocorticoid receptors in human vascular endothelial cells regulate intercellular adhesion molecule-1 expression and promote leukocyte adhesion. Circ Res. 2008;102(11):1359-67.

74. Schiffrin EL, Franks DJ, Gutkowska J. Effect of aldosterone on vascular angiotensin II receptors in the rat. Can J Physiol Pharmacol. 1985;63(12):1522-7.

75. Min LJ, Mogi M, Li JM, Iwanami J, Iwai M, Horiuchi M. Aldosterone and angiotensin II synergistically induce mitogenic response in vascular smooth muscle cells. Circ Res. 2005;97(5):434-42.

76. Montezano AC, Callera GE, Yogi A, He Y, Tostes RC, He G, et al. Aldosterone and angiotensin II synergistically stimulate migration in vascular smooth muscle cells through c-Src-regulated redox-sensitive RhoA pathways. Arterioscler Thromb Vasc Biol. 2008;28(8):1511-8.

77. Batenburg WW, Jansen PM, van den Bogaerdt AJ, AH JD. Angiotensin II-aldosterone interaction in human coronary microarteries involves GPR30, EGFR, and endothelial NO synthase. Cardiovasc Res. 2012;94(1):136-43.

78. Min LJ, Mogi M, Iwanami J, Li JM, Sakata A, Fujita T, et al. Cross-talk between aldosterone and angiotensin II in vascular smooth muscle cell senescence. Cardiovasc Res. 2007;76(3):506-16.

79. Wehling M, Spes CH, Win N, Janson CP, Schmidt BM, Theisen K, et al. Rapid cardiovascular action of aldosterone in man. J Clin Endocrinol Metab. 1998;83(10):3517-22.

80. Gros R, Ding Q, Sklar LA, Prossnitz EE, Arterburn JB, Chorazyczewski J, et al. GPR30 expression is required for the mineralocorticoid receptor-independent rapid vascular effects of aldosterone. Hypertension. 2011;57(3):442-51.

81. Gros R, Ding Q, Liu B, Chorazyczewski J, Feldman RD. Aldosterone mediates its rapid effects in vascular endothelial cells through GPER activation. Am J Physiol Cell Physiol. 2013;304(6):C532-40.

82. Briet M, Schiffrin EL. Vascular actions of aldosterone. J Vasc Res. 2013;50(2):89-99.

83. Feldman RD, Gros R. Vascular effects of aldosterone: sorting out the receptors and the ligands. Clin Exp Pharmacol Physiol. 2013;40(12):916-21.

84. Hussain Y, Ding Q, Connelly PW, Brunt JH, Ban MR, McIntyre AD, et al. G-protein estrogen receptor as a regulator of low-density lipoprotein cholesterol metabolism: cellular and population genetic studies. Arterioscler Thromb Vasc Biol. 2015;35(1):213-21.

85. Briet M, Barhoumi T, Mian MO, Coelho SC, Ouerd S, Rautureau Y, et al. Aldosterone-Induced Vascular Remodeling and Endothelial Dysfunction Require Functional Angiotensin Type 1a Receptors. Hypertension. 2016;67(5):897-905.

86. Ingelsson E, Pencina MJ, Tofler GH, Benjamin EJ, Lanier KJ, Jacques PF, et al. Multimarker approach to evaluate the incidence of the metabolic syndrome and longitudinal changes in metabolic risk factors: the Framingham Offspring Study. Circulation. 2007;116(9):984-92.

87. Bentley-Lewis R, Adler GK, Perlstein T, Seely EW, Hopkins PN, Williams GH, et al. Body mass index predicts aldosterone production in normotensive adults on a high-salt diet. J Clin Endocrinol Metab. 2007;92(11):4472-5.

88. Goodfriend TL, Kelley DE, Goodpaster BH, Winters SJ. Visceral obesity and insulin resistance are associated with plasma aldosterone levels in women. Obes Res. 1999;7(4):355-62.

89. Briones AM, Nguyen Dinh Cat A, Callera GE, Yogi A, Burger D, He Y, et al. Adipocytes produce aldosterone through calcineurin-dependent signaling pathways: implications in diabetes mellitus-associated obesity and vascular dysfunction. Hypertension. 2012;59(5):1069-78.

90. Nguyen G, Contrepas A. The (pro)renin receptors. J Mol Med (Berl). 2008;86(6):643-6.

91. Brown NJ, Agirbasli M, Vaughan DE. Comparative effect of angiotensin-converting enzyme inhibition and angiotensin II type 1 receptor antagonism on plasma fibrinolytic balance in humans. Hypertension. 1999;34(2):285-90.

92. Hitom H, Liu G, Nishiyama A. Role of (pro)renin receptor in cardiovascular cells from the aspect of signaling. Front Biosci (Elite Ed). 2010;2:1246-9.

93. Lastra G, Habibi J, Whaley-Connell AT, Manrique C, Hayden MR, Rehmer J, et al. Direct renin inhibition improves systemic insulin resistance and skeletal muscle glucose transport in a transgenic rodent model of tissue renin overexpression. Endocrinology. 2009;150(6):2561-8.

94. Nguyen G, Muller DN. The biology of the (pro)renin receptor. J Am Soc Nephrol. 2010;21(1):18-23.

95. Miyazaki M, Takai S, Jin D, Muramatsu M. Pathological roles of angiotensin II produced by mast cell chymase and the effects of chymase inhibition in animal models. Pharmacol Ther. 2006;112(3):668-76.

96. Rykl J, Thiemann J, Kurzawski S, Pohl T, Gobom J, Zidek W, et al. Renal cathepsin G and angiotensin II generation. J Hypertens. 2006;24(9):1797-807.

97. Padmanabhan N, Jardine AG, McGrath JC, Connell JM. Angiotensin-converting enzyme-independent contraction to angiotensin I in human resistance arteries. Circulation. 1999;99(22):2914-20.

98. Hollenberg NK, Fisher ND, Price DA. Pathways for angiotensin II generation in intact human tissue: evidence from comparative pharmacological interruption of the renin system. Hypertension. 1998;32(3):387-92.

99. Nickenig G. Central role of the AT(1)-receptor in atherosclerosis. J Hum Hypertens. 2002;16 Suppl 3:S26-33.

100. Griendling KK, Ushio-Fukai M, Lassegue B, Alexander RW. Angiotensin II signaling in vascular smooth muscle. New concepts. Hypertension. 1997;29(1 Pt 2):366-73.

101. Nguyen Dinh Cat A, Montezano AC, Burger D, Touyz RM. Angiotensin II, NADPH oxidase, and redox signaling in the vasculature. Antioxid Redox Signal. 2013;19(10):1110-20.

102. Touyz RM, Cruzado M, Tabet F, Yao G, Salomon S, Schiffrin EL. Redox-dependent MAP kinase signaling by Ang II in vascular smooth muscle cells: role of receptor tyrosine kinase transactivation. Can J Physiol Pharmacol. 2003;81(2):159-67.

103. Loirand G, Pacaud P. The role of Rho protein signaling in hypertension. Nat Rev Cardiol. 2010;7(11):637-47.

104. Brasier AR, Recinos A, 3rd, Eledrisi MS. Vascular inflammation and the renin-angiotensin system. Arterioscler Thromb Vasc Biol. 2002;22(8):1257-66.

105. Itoh H, Mukoyama M, Pratt RE, Gibbons GH, Dzau VJ. Multiple autocrine growth factors modulate vascular smooth muscle cell growth response to angiotensin II. J Clin Invest. 1993;91(5):2268-74.

106. Schieffer B, Schieffer E, Hilfiker-Kleiner D, Hilfiker A, Kovanen PT, Kaartinen M, et al. Expression of angiotensin II and interleukin 6 in human coronary atherosclerotic plaques: potential implications for inflammation and plaque instability. Circulation. 2000;101(12):1372-8.

107. Pollman MJ, Yamada T, Horiuchi M, Gibbons GH. Vasoactive substances regulate vascular smooth muscle cell apoptosis. Countervailing influences of nitric oxide and angiotensin II. Circ Res. 1996;79(4):748-56.

108. Mackie EJ, Scott-Burden T, Hahn AW, Kern F, Bernhardt J, Regenass S, et al. Expression of tenascin by vascular smooth muscle cells. Alterations in hypertensive rats and stimulation by angiotensin II. Am J Pathol. 1992;141(2):377-88.

109. Cifuentes-Pagano E, Meijles DN, Pagano PJ. The quest for selective nox inhibitors and therapeutics: challenges, triumphs and pitfalls. Antioxid Redox Signal. 2014;20(17):2741-54.

110. Matchar DB, McCrory DC, Orlando LA, Patel MR, Patel UD, Patwardhan MB, et al. Systematic review: comparative effectiveness of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers for treating essential hypertension. Ann Intern Med. 2008;148(1):16-29.

111. Antony I, Lerebours G, Nitenberg A. Angiotensin-converting enzyme inhibition restores flow-dependent and cold pressor test-induced dilations in coronary arteries of hypertensive patients. Circulation. 1996;94(12):3115-22.

112. Mancini GB, Henry GC, Macaya C, O'Neill BJ, Pucillo AL, Carere RG, et al. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease. The TREND (Trial on Reversing ENdothelial Dysfunction) Study. Circulation. 1996;94(3):258-65.

113. Fox KM. Efficacy of perindopril in reduction of cardiovascular events among patients with stable coronary artery disease: randomised, double-blind, placebo-controlled, multicentre trial (the EUROPA study). Lancet. 2003;362(9386):782-8.

114. Braunwald E, Domanski MJ, Fowler SE, Geller NL, Gersh BJ, Hsia J, et al. Angiotensin-converting-enzyme inhibition in stable coronary artery disease. N Engl J Med. 2004;351(20):2058-68.

115. Pitt B, O'Neill B, Feldman R, Ferrari R, Schwartz L, Mudra H, et al. The QUinapril Ischemic Event Trial (QUIET): evaluation of chronic ACE inhibitor therapy in patients with ischemic heart disease and preserved left ventricular function. Am J Cardiol. 2001;87(9):1058-63.

116. Folkow B. Physiological aspects of primary hypertension. Physiol Rev. 1982;62(2):347-504.

117. Feihl F, Liaudet L, Levy BI, Waeber B. Hypertension and microvascular remodelling. Cardiovasc Res. 2008;78(2):274-85.

118. Levy BI, Schiffrin EL, Mourad JJ, Agostini D, Vicaut E, Safar ME, et al. Impaired tissue perfusion: a pathology common to hypertension, obesity, and diabetes mellitus. Circulation. 2008;118(9):968-76.

119. Chae CU, Lee RT, Rifai N, Ridker PM. Blood pressure and inflammation in apparently healthy men. Hypertension. 2001;38(3):399-403.

120. Tang EH, Vanhoutte PM. Prostanoids and reactive oxygen species: team players in endothelium-dependent contractions. Pharmacol Ther. 2009;122(2):140-9.

121. Hernanz R, Briones AM, Salaices M, Alonso MJ. New roles for old pathways? A circuitous relationship between reactive oxygen species and cyclo-oxygenase in hypertension. Clin Sci (Lond). 2014;126(2):111-21.

122. Feletou M, Vanhoutte PM. Endothelial dysfunction: a multifaceted disorder (The Wiggers Award Lecture). Am J Physiol Heart Circ Physiol. 2006;291(3):H985-1002.

123. Vanhoutte PM, Shimokawa H, Tang EH, Feletou M. Endothelial dysfunction and vascular disease. Acta Physiol (Oxf). 2009;196(2):193-222.

124. Chien S. Mechanotransduction and endothelial cell homeostasis: the wisdom of the cell. Am J Physiol Heart Circ Physiol. 2007;292(3):H1209-24.

125. Chiu JJ, Chien S. Effects of disturbed flow on vascular endothelium: pathophysiological basis and clinical perspectives. Physiol Rev. 2011;91(1):327-87.

126. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci U S A. 1987;84(24):9265-9.

127. Rochier A, Nixon A, Yamashita N, Abe R, Abe R, Madri JA, et al. Laminar shear, but not orbital shear, has a synergistic effect with thrombin stimulation on tissue factor expression in human umbilical vein endothelial cells. J Vasc Surg. 2011;54(2):480-8.

128. Kawai Y, Matsumoto Y, Watanabe K, Yamamoto H, Satoh K, Murata M, et al. Hemodynamic forces modulate the effects of cytokines on fibrinolytic activity of endothelial cells. Blood. 1996;87(6):2314-21.

129. Takada Y, Shinkai F, Kondo S, Yamamoto S, Tsuboi H, Korenaga R, et al. Fluid shear stress increases the expression of thrombomodulin by cultured human endothelial cells. Biochem Biophys Res Commun. 1994;205(2):1345-52.

130. Ramkhelawon B, Vilar J, Rivas D, Mees B, de Crom R, Tedgui A, et al. Shear stress regulates angiotensin type 1 receptor expression in endothelial cells. Circ Res. 2009;105(9):869-75.

131. Welch WJ. Angiotensin II-dependent superoxide: effects on hypertension and vascular dysfunction. Hypertension. 2008;52(1):51-6.

132. Godbole AS, Lu X, Guo X, Kassab GS. NADPH oxidase has a directional response to shear stress. Am J Physiol Heart Circ Physiol. 2009;296(1):H152-8.

133. Zou MH. Peroxynitrite and protein tyrosine nitration of prostacyclin synthase. Prostaglandins Other Lipid Mediat. 2007;82(1-4):119-27.

134. Kohnen SL, Mouithys-Mickalad AA, Deby-Dupont GP, Deby CM, Lamy ML, Noels AF. Oxidation of tetrahydrobiopterin by peroxynitrite or oxoferryl species occurs by a radical pathway. Free Radic Res. 2001;35(6):709-21.

135. Delli Gatti C, Osto E, Kouroedov A, Eto M, Shaw S, Volpe M, et al. Pulsatile stretch induces release of angiotensin II and oxidative stress in human endothelial cells: effects of ACE inhibition and AT1 receptor antagonism. Clin Exp Hypertens. 2008;30(7):616-27.

136. Zou Y, Akazawa H, Qin Y, Sano M, Takano H, Minamino T, et al. Mechanical stress activates angiotensin II type 1 receptor without the involvement of angiotensin II. Nat Cell Biol. 2004;6(6):499-506.

137. Schwartz MA. Integrins and extracellular matrix in mechanotransduction. Cold Spring Harb Perspect Biol. 2010;2(12):a005066.

138. Greene AS, Tonellato PJ, Lui J, Lombard JH, Cowley AW, Jr. Microvascular rarefaction and tissue vascular resistance in hypertension. Am J Physiol. 1989;256(1 Pt 2):H126-31.

139. Battegay EJ, de Miguel LS, Petrimpol M, Humar R. Effects of anti-hypertensive drugs on vessel rarefaction. Curr Opin Pharmacol. 2007;7(2):151-7.

140. Boulanger CM. Secondary endothelial dysfunction: hypertension and heart failure. J Mol Cell Cardiol. 1999;31(1):39-49.

141. Edelberg JM, Reed MJ. Aging and angiogenesis. Front Biosci. 2003;8:s1199-209.

142. Kiefer FN, Misteli H, Kalak N, Tschudin K, Fingerle J, Van der Kooij M, et al. Inhibition of NO biosynthesis, but not elevated blood pressure, reduces angiogenesis in rat models of secondary hypertension. Blood Press. 2002;11(2):116-24.

143. Vilar J, Waeckel L, Bonnin P, Cochain C, Loinard C, Duriez M, et al. Chronic hypoxia-induced angiogenesis normalizes blood pressure in spontaneously hypertensive rats. Circ Res. 2008;103(7):761-9.

144. Luque Contreras D, Vargas Robles H, Romo E, Rios A, Escalante B. The role of nitric oxide in the post-ischemic revascularization process. Pharmacol Ther. 2006;112(2):553-63.

145. Debbabi H, Uzan L, Mourad JJ, Safar M, Levy BI, Tibirica E. Increased skin capillary density in treated essential hypertensive patients. Am J Hypertens. 2006;19(5):477-83.

146. Hughes AD, Stanton AV, Jabbar AS, Chapman N, Martinez-Perez ME, Mc GTSA. Effect of antihypertensive treatment on retinal microvascular changes in hypertension. J Hypertens. 2008;26(8):1703-7.

147. Schiffrin EL, Deng LY, Larochelle P. Effects of a beta-blocker or a converting enzyme inhibitor on resistance arteries in essential hypertension. Hypertension. 1994;23(1):83-91.

148. Ichioka S, Shibata M, Kosaki K, Sato Y, Harii K, Kamiya A. In vivo measurement of morphometric and hemodynamic changes in the microcirculation during angiogenesis under chronic alpha1-adrenergic blocker treatment. Microvasc Res. 1998;55(2):165-74.

149. Fulgenzi G, Graciotti L, Collis MG, Hudlicka O. The effect of alpha 1 adrenoceptor antagonist prazosin on capillary supply, blood flow and performance in a rat model of chronic muscle ischaemia. Eur J Vasc Endovasc Surg. 1998;16(1):71-7.

150. Keledjian K, Garrison JB, Kyprianou N. Doxazosin inhibits human vascular endothelial cell adhesion, migration, and invasion. J Cell Biochem. 2005;94(2):374-88.

151. Olsen MH, Fossum E, Hoieggen A, Wachtell K, Hjerkinn E, Nesbitt SD, et al. Long-term treatment with losartan versus atenolol improves insulin sensitivity in hypertension: ICARUS, a LIFE substudy. J Hypertens. 2005;23(4):891-8.

152. Rodrigo R, Libuy M, Feliu F, Hasson D. Oxidative stress-related biomarkers in essential hypertension and ischemia-reperfusion myocardial damage. Dis Markers. 2013;35(6):773-90.

153. Touyz RM, Yao G, Quinn MT, Pagano PJ, Schiffrin EL. p47phox associates with the cytoskeleton through cortactin in human vascular smooth muscle cells: role in NAD(P)H oxidase regulation by angiotensin II. Arterioscler Thromb Vasc Biol. 2005;25(3):512-8.

154. Touyz RM, Schiffrin EL. Increased generation of superoxide by angiotensin II in smooth muscle cells from resistance arteries of hypertensive patients: role of phospholipase D-dependent NAD(P)H oxidase-sensitive pathways. J Hypertens. 2001;19(7):1245-54.

155. Ghiadoni L, Magagna A, Versari D, Kardasz I, Huang Y, Taddei S, et al. Different effect of antihypertensive drugs on conduit artery endothelial function. Hypertension. 2003;41(6):1281-6.

156. Anderson TJ, Elstein E, Haber H, Charbonneau F. Comparative study of ACE-inhibition, angiotensin II antagonism, and calcium channel blockade on flow-mediated vasodilation in patients with coronary disease (BANFF study). J Am Coll Cardiol. 2000;35(1):60-6.

157. Candido R, Allen TJ, Lassila M, Cao Z, Thallas V, Cooper ME, et al. Irbesartan but not amlodipine suppresses diabetes-associated atherosclerosis. Circulation. 2004;109(12):1536-42.

158. Doran DE, Weiss D, Zhang Y, Griendling KK, Taylor WR. Differential effects of AT1 receptor and Ca2+ channel blockade on atherosclerosis, inflammatory gene expression, and production of reactive oxygen species. Atherosclerosis. 2007;195(1):39-47.

159. Hirooka Y, Kimura Y, Sagara Y, Ito K, Sunagawa K. Effects of valsartan or amlodipine on endothelial function and oxidative stress after one year follow-up in patients with essential hypertension. Clin Exp Hypertens. 2008;30(3):267-76.

160. Schwieler JH, Kahan T, Wallen NH, Nussberger J, Hjemdahl P. Inhibition of the renin-angiotensin system does not reduce platelet activity at rest or during stress in hypertension. J Hypertens. 2013;31(8):1676-82.

161. McNeely MJ, Edwards KL, Marcovina SM, Brunzell JD, Motulsky AG, Austin MA. Lipoprotein and apolipoprotein abnormalities in familial combined hyperlipidaemia: a 20-year prospective study. Atherosclerosis. 2001;159(2):471-81.

162. Naukkarinen J, Ehnholm C, Peltonen L. Genetics of familial combined hyperlipidaemia. Curr Opin Lipidol. 2006;17(3):285-90.

163. Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidaemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidaemia. J Clin Invest. 1973;52(7):1544-68.

164. Austin MA, McKnight B, Edwards KL, Bradley CM, McNeely MJ, Psaty BM, et al. Cardiovascular disease mortality in familial forms of hypertriglyceridemia: A 20-year prospective study. Circulation. 2000;101(24):2777-82.

165. Liu ML, Ylitalo K, Nuotio I, Salonen R, Salonen JT, Taskinen MR. Association between carotid intima-media thickness and low-density lipoprotein size and susceptibility of low-density lipoprotein to oxidation in asymptomatic members of familial combined hyperlipidaemia families. Stroke. 2002;33(5):1255-60.

166. Olufadi R, Byrne CD. Effects of VLDL and remnant particles on platelets. Pathophysiol Haemost Thromb. 2006;35(3-4):281-91.

167. Georgieva AM, Cate HT, Keulen ET, van Oerle R, Govers-Riemslag JW, Hamulyak K, et al. Prothrombotic markers in familial combined hyperlipidaemia: evidence of endothelial cell activation and relation to metabolic syndrome. Atherosclerosis. 2004;175(2):345-51.

168. Austin MA, Hutter CM, Zimmern RL, Humphries SE. Familial hypercholesterolemia and coronary heart disease: a HuGE association review. Am J Epidemiol. 2004;160(5):421-9.

169. Kaul S, Waack BJ, Padgett RC, Brooks RM, Heistad DD. Altered vascular responses to platelets from hypercholesterolemic humans. Circ Res. 1993;72(4):737-43.

170. Owens AP, 3rd, Passam FH, Antoniak S, Marshall SM, McDaniel AL, Rudel L, et al. Monocyte tissue factor-dependent activation of coagulation in hypercholesterolemic mice and monkeys is inhibited by simvastatin. J Clin Invest. 2012;122(2):558-68.

171. Sambola A, Osende J, Hathcock J, Degen M, Nemerson Y, Fuster V, et al. Role of risk factors in the modulation of tissue factor activity and blood thrombogenicity. Circulation. 2003;107(7):973-7.

172. Catar RA, Muller G, Heidler J, Schmitz G, Bornstein SR, Morawietz H. Low-density lipoproteins induce the renin-angiotensin system and their receptors in human endothelial cells. Horm Metab Res. 2007;39(11):801-5.

173. Lloyd-Jones DM, Evans JC, Larson MG, O'Donnell CJ, Wilson PW, Levy D. Cross-classification of JNC VI blood pressure stages and risk groups in the Framingham Heart Study. Arch Intern Med. 1999;159(18):2206-12.

174. Panz V, Immelman A, Paiker J, Pilcher G, Raal F. High-dose statin therapy does not induce insulin resistance in patients with familial hypercholesterolemia. Metab Syndr Relat Disord. 2012;10(5):351-7.

175. Tabas I, Williams KJ, Boren J. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. Circulation. 2007;116(16):1832-44.

176. Rios FJ, Koga MM, Pecenin M, Ferracini M, Gidlund M, Jancar S. Oxidized LDL induces alternative macrophage phenotype through activation of CD36 and PAFR. Mediators Inflamm. 2013;2013:198193.

177. Libby P, Lichtman AH, Hansson GK. Immune effector mechanisms implicated in atherosclerosis: from mice to humans. Immunity. 2013;38(6):1092-104.

178. Kiyan Y, Tkachuk S, Hilfiker-Kleiner D, Haller H, Fuhrman B, Dumler I. oxLDL induces inflammatory responses in vascular smooth muscle cells via urokinase receptor association with CD36 and TLR4. J Mol Cell Cardiol. 2014;66:72-82.

179. Hu C, Dandapat A, Mehta JL. Angiotensin II induces capillary formation from endothelial cells via the LOX-1 dependent redox-sensitive pathway. Hypertension. 2007;50(5):952-7.

180. Taye A, Saad AH, Kumar AH, Morawietz H. Effect of apocynin on NADPH oxidase-mediated oxidative stress-LOX-1-eNOS pathway in human endothelial cells exposed to high glucose. Eur J Pharmacol. 2010;627(1-3):42-8.

181. Lusis AJ. Atherosclerosis. Nature. 2000;407(6801):233-41.

182. Shepherd PR, Kahn BB. Glucose transporters and insulin action--implications for insulin resistance and diabetes mellitus. N Engl J Med. 1999;341(4):248-57.

183. Nistala R, Stump CS. Skeletal muscle insulin resistance is fundamental to the cardiometabolic syndrome. J Cardiometab Syndr. 2006;1(1):47-52.

184. McMurray JJ, Holman RR, Haffner SM, Bethel MA, Holzhauer B, Hua TA, et al. Effect of valsartan on the incidence of diabetes and cardiovascular events. N Engl J Med. 2010;362(16):1477-90.

185. Dahlof B, Devereux RB, Kjeldsen SE, Julius S, Beevers G, de Faire U, et al. Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. Lancet. 2002;359(9311):995-1003.

186. Abuissa H, Jones PG, Marso SP, O'Keefe JH, Jr. Angiotensin-converting enzyme inhibitors or angiotensin receptor blockers for prevention of type 2 diabetes: a meta-analysis of randomized clinical trials. J Am Coll Cardiol. 2005;46(5):821-6.

187. Prasannarong M, Santos FR, Henriksen EJ. ANG-(1-7) reduces ANG II-induced insulin resistance by enhancing Akt phosphorylation via a Mas receptor-dependent mechanism in rat skeletal muscle. Biochem Biophys Res Commun. 2012;426(3):369-73.

188. Henriksen EJ, Prasannarong M. The role of the renin-angiotensin system in the development of insulin resistance in skeletal muscle. Mol Cell Endocrinol. 2013;378(1-2):15-22.

189. Feldman RD, Schmidt ND. Moderate dietary salt restriction increases vascular and systemic insulin resistance. Am J Hypertens. 1999;12(6):643-7.

190. Luther JM, Luo P, Wang Z, Cohen SE, Kim HS, Fogo AB, et al. Aldosterone deficiency and mineralocorticoid receptor antagonism prevent angiotensin II-induced cardiac, renal, and vascular injury. Kidney Int. 2012;82(6):643-51.

191. Hitomi H, Kiyomoto H, Nishiyama A, Hara T, Moriwaki K, Kaifu K, et al. Aldosterone suppresses insulin signaling via the downregulation of insulin receptor substrate-1 in vascular smooth muscle cells. Hypertension. 2007;50(4):750-5.

192. Luther JM, Luo P, Kreger MT, Brissova M, Dai C, Whitfield TT, et al. Aldosterone decreases glucose-stimulated insulin secretion in vivo in mice and in murine islets. Diabetologia. 2011;54(8):2152-63.

193. Fogari R, Zoppi A, Mugellini A, Lazzari P, Derosa G. Different effects of aliskiren and losartan on fibrinolysis and insulin sensitivity in hypertensive patients with metabolic syndrome. Horm Metab Res. 2010;42(12):892-6.

194. Davizon P, Lopez JA. Microparticles and thrombotic disease. Curr Opin Hematol. 2009;16(5):334-41.

195. Egorina EM, Sovershaev MA, Olsen JO, Osterud B. Granulocytes do not express but acquire monocyte-derived tissue factor in whole blood: evidence for a direct transfer. Blood. 2008;111(3):1208-16.

196. Bouchard BA, Mann KG, Butenas S. No evidence for tissue factor on platelets. Blood. 2010;116(5):854-5.

197. Camera M, Brambilla M, Toschi V, Tremoli E. Tissue factor expression on platelets is a dynamic event. Blood. 2010;116(23):5076-7.

198. Bogdanov VY, Balasubramanian V, Hathcock J, Vele O, Lieb M, Nemerson Y. Alternatively spliced human tissue factor: a circulating, soluble, thrombogenic protein. Nat Med. 2003;9(4):458-62.

199. Balasubramanian V, Grabowski E, Bini A, Nemerson Y. Platelets, circulating tissue factor, and fibrin colocalize in ex vivo thrombi: real-time fluorescence images of thrombus formation and propagation under defined flow conditions. Blood. 2002;100(8):2787-92.

200. Rao LV, Kothari H, Pendurthi UR. Tissue factor encryption and decryption: facts and controversies. Thromb Res. 2012;129 Suppl 2:S13-7.

201. van der Poll T, Levi M, Hack CE, ten Cate H, van Deventer SJ, Eerenberg AJ, et al. Elimination of interleukin 6 attenuates coagulation activation in experimental endotoxemia in chimpanzees. J Exp Med. 1994;179(4):1253-9.

202. Levi M, van der Poll T, ten Cate H, van Deventer SJ. The cytokine-mediated imbalance between coagulant and anticoagulant mechanisms in sepsis and endotoxaemia. Eur J Clin Invest. 1997;27(1):3-9.

203. Cirillo P, Golino P, Calabro P, Cali G, Ragni M, De Rosa S, et al. C-reactive protein induces tissue factor expression and promotes smooth muscle and endothelial cell proliferation. Cardiovasc Res. 2005;68(1):47-55.

204. Cirillo P, Cali G, Golino P, Calabro P, Forte L, De Rosa S, et al. Tissue factor binding of activated factor VII triggers smooth muscle cell proliferation via extracellular signal-regulated kinase activation. Circulation. 2004;109(23):2911-6.

205. Hoffman M, Monroe DM, 3rd. A cell-based model of hemostasis. Thromb Haemost. 2001;85(6):958-65.

206. Wu Y. Contact pathway of coagulation and inflammation. Thromb J. 2015;13:17.

207. Zhu D. Mathematical modeling of blood coagulation cascade: kinetics of intrinsic and extrinsic pathways in normal and deficient conditions. Blood Coagul Fibrinolysis. 2007;18(7):637-46.

208. Lenting PJ, van Mourik JA, Mertens K. The life cycle of coagulation factor VIII in view of its structure and function. Blood. 1998;92(11):3983-96.

209. Bouma BN, Mosnier LO. Thrombin activatable fibrinolysis inhibitor (TAFI)--how does thrombin regulate fibrinolysis? Ann Med. 2006;38(6):378-88.

210. Esmon CT. The protein C pathway. Chest. 2003;124(3 Suppl):26s-32s.

211. Brass LF. Thrombin and platelet activation. Chest. 2003;124(3 Suppl):18s-25s.

212. Sapet C, Simoncini S, Loriod B, Puthier D, Sampol J, Nguyen C, et al. Thrombin-induced endothelial microparticle generation: identification of a novel pathway involving ROCK-II activation by caspase-2. Blood. 2006;108(6):1868-76.

213. Gudmundsdottir IJ, Megson IL, Kell JS, Ludlam CA, Fox KA, Webb DJ, et al. Direct vascular effects of protease-activated receptor type 1 agonism in vivo in humans. Circulation. 2006;114(15):1625-32.

214. Hirano K. The roles of proteinase-activated receptors in the vascular physiology and pathophysiology. Arterioscler Thromb Vasc Biol. 2007;27(1):27-36.

215. Rahman A, Anwar KN, True AL, Malik AB. Thrombin-induced p65 homodimer binding to downstream NF-kappa B site of the promoter mediates endothelial ICAM-1 expression and neutrophil adhesion. J Immunol. 1999;162(9):5466-76.

216. Marin V, Montero-Julian FA, Gres S, Boulay V, Bongrand P, Farnarier C, et al. The IL-6-soluble IL-6Ralpha autocrine loop of endothelial activation as an intermediate between acute and chronic inflammation: an experimental model involving thrombin. J Immunol. 2001;167(6):3435-42.

217. Bajzar L, Morser J, Nesheim M. TAFI, or plasma procarboxypeptidase B, couples the coagulation and fibrinolytic cascades through the thrombin-thrombomodulin complex. J Biol Chem. 1996;271(28):16603-8.

218. Davidson SJ. Inflammation and Acute Phase Proteins in Haemostasis. In: Janciauskiene S, editor. Acute Phase Proteins. Rijeka: InTech; 2013. p. Ch. 02.

219. Aso Y. Plasminogen activator inhibitor (PAI)-1 in vascular inflammation and thrombosis. Front Biosci. 2007;12:2957-66.

220. Brown NJ, Vaughan DE. Prothrombotic effects of angiotensin. Adv Intern Med. 2000;45:419-29.

221. van Leeuwen RT, Kol A, Andreotti F, Kluft C, Maseri A, Sperti G. Angiotensin II increases plasminogen activator inhibitor type 1 and tissue-type plasminogen

activator messenger RNA in cultured rat aortic smooth muscle cells. Circulation. 1994;90(1):362-8.

222. Kerins DM, Hao Q, Vaughan DE. Angiotensin induction of PAI-1 expression in endothelial cells is mediated by the hexapeptide angiotensin IV. J Clin Invest. 1995;96(5):2515-20.

223. Boman KO, Jansson JH, Nyhlen KA, Nilsson TK. Improved fibrinolysis after one year of treatment with enalapril in men and women with uncomplicated myocardial infarction. Thromb Haemost. 2002;87(2):311-6.

224. Nakamura S, Nakamura I, Ma L, Vaughan DE, Fogo AB. Plasminogen activator inhibitor-1 expression is regulated by the angiotensin type 1 receptor in vivo. Kidney Int. 2000;58(1):251-9.

225. Ridker PM, Gaboury CL, Conlin PR, Seely EW, Williams GH, Vaughan DE. Stimulation of plasminogen activator inhibitor in vivo by infusion of angiotensin II. Evidence of a potential interaction between the renin-angiotensin system and fibrinolytic function. Circulation. 1993;87(6):1969-73.

226. Lottermoser K, Hertfelder HJ, Gohlke P, Vetter H, Dusing R. Short-term effects of exogenous angiotensin II on plasma fibrinolytic balance in normal subjects. Clin Exp Hypertens. 2004;26(1):13-26.

227. Larsson PT, Schwieler JH, Wallen NH, Hjemdahl P. Acute effects of angiotensin II on fibrinolysis in healthy volunteers. Blood Coagul Fibrinolysis. 1999;10(1):19-24.

228. Verhamme P, Hoylaerts MF. Hemostasis and inflammation: two of a kind? Thromb J. 2009;7:15.

229. Ceciliani F, Giordano A, Spagnolo V. The systemic reaction during inflammation: the acute-phase proteins. Protein Pept Lett. 2002;9(3):211-23.

230. Zimmerman GA, McIntyre TM, Prescott SM, Stafforini DM. The platelet-activating factor signaling system and its regulators in syndromes of inflammation and thrombosis. Crit Care Med. 2002;30(5 Suppl):S294-301.

231. Kaser A, Brandacher G, Steurer W, Kaser S, Offner FA, Zoller H, et al. Interleukin-6 stimulates thrombopoiesis through thrombopoietin: role in inflammatory thrombocytosis. Blood. 2001;98(9):2720-5.

232. Kahn ML, Nakanishi-Matsui M, Shapiro MJ, Ishihara H, Coughlin SR. Protease-activated receptors 1 and 4 mediate activation of human platelets by thrombin. J Clin Invest. 1999;103(6):879-87.

233. Polgar J, Matuskova J, Wagner DD. The P-selectin, tissue factor, coagulation triad. J Thromb Haemost. 2005;3(8):1590-6.

234. Gawaz M, Page S, Massberg S, Nothdurfter C, Weber M, Fisher C, et al. Transient platelet interaction induces MCP-1 production by endothelial cells via I kappa B kinase complex activation. Thromb Haemost. 2002;88(2):307-14.

235. Gawaz M, Brand K, Dickfeld T, Pogatsa-Murray G, Page S, Bogner C, et al. Platelets induce alterations of chemotactic and adhesive properties of endothelial cells mediated through an interleukin-1-dependent mechanism. Implications for atherogenesis. Atherosclerosis. 2000;148(1):75-85.

236. Mach F, Schonbeck U, Sukhova GK, Bourcier T, Bonnefoy JY, Pober JS, et al. Functional CD40 ligand is expressed on human vascular endothelial cells, smooth

muscle cells, and macrophages: implications for CD40-CD40 ligand signaling in atherosclerosis. Proc Natl Acad Sci U S A. 1997;94(5):1931-6.

237. Antoniades C, Bakogiannis C, Tousoulis D, Antonopoulos AS, Stefanadis C. The CD40/CD40 ligand system: linking inflammation with atherothrombosis. J Am Coll Cardiol. 2009;54(8):669-77.

238. Osterud B, Olsen JO. Human platelets do not express tissue factor. Thromb Res. 2013;132(1):112-5.

239. Mallat Z, Benamer H, Hugel B, Benessiano J, Steg PG, Freyssinet JM, et al. Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. Circulation. 2000;101(8):841-3.

240. Cimmino G, D'Amico C, Vaccaro V, D'Anna M, Golino P. The missing link between atherosclerosis, inflammation and thrombosis: is it tissue factor? Expert Rev Cardiovasc Ther. 2011;9(4):517-23.

241. Brand K, Banka CL, Mackman N, Terkeltaub RA, Fan ST, Curtiss LK. Oxidized LDL enhances lipopolysaccharide-induced tissue factor expression in human adherent monocytes. Arterioscler Thromb. 1994;14(5):790-7.

242. Golino P, Ragni M, Cirillo P, Avvedimento VE, Feliciello A, Esposito N, et al. Effects of tissue factor induced by oxygen free radicals on coronary flow during reperfusion. Nat Med. 1996;2(1):35-40.

243. Esmon CT. Inflammation and thrombosis. J Thromb Haemost. 2003;1(7):1343-8.

244. Esmon CT. The impact of the inflammatory response on coagulation. Thromb Res. 2004;114(5-6):321-7.

245. Ostrovsky L, Woodman RC, Payne D, Teoh D, Kubes P. Antithrombin III prevents and rapidly reverses leukocyte recruitment in ischemia/reperfusion. Circulation. 1997;96(7):2302-10.

246. Bourin MC, Lindahl U. Glycosaminoglycans and the regulation of blood coagulation. Biochem J. 1993;289 (Pt 2):313-30.

247. Laszik Z, Mitro A, Taylor FB, Jr., Ferrell G, Esmon CT. Human protein C receptor is present primarily on endothelium of large blood vessels: implications for the control of the protein C pathway. Circulation. 1997;96(10):3633-40.

248. Esmon CT. The endothelial cell protein C receptor. Thromb Haemost. 2000;83(5):639-43.

249. Rezaie AR. Vitronectin functions as a cofactor for rapid inhibition of activated protein C by plasminogen activator inhibitor-1. Implications for the mechanism of profibrinolytic action of activated protein C. J Biol Chem. 2001;276(19):15567-70.

250. Conway EM, Van de Wouwer M, Pollefeyt S, Jurk K, Van Aken H, De Vriese A, et al. The lectin-like domain of thrombomodulin confers protection from neutrophil-mediated tissue damage by suppressing adhesion molecule expression via nuclear factor kappaB and mitogen-activated protein kinase pathways. J Exp Med. 2002;196(5):565-77.

251. Kurosawa S, Esmon CT, Stearns-Kurosawa DJ. The soluble endothelial protein C receptor binds to activated neutrophils: involvement of proteinase-3 and CD11b/CD18. J Immunol. 2000;165(8):4697-703.

252. Conway EM, Rosenberg RD. Tumor necrosis factor suppresses transcription of the thrombomodulin gene in endothelial cells. Mol Cell Biol. 1988;8(12):5588-92.

253. Takano S, Kimura S, Ohdama S, Aoki N. Plasma thrombomodulin in health and diseases. Blood. 1990;76(10):2024-9.

254. Cimmino G, Cirillo P, Ragni M, Conte S, Uccello G, Golino P. Reactive oxygen species induce a procoagulant state in endothelial cells by inhibiting tissue factor pathway inhibitor. J Thromb Thrombolysis. 2015;40(2):186-92.

255. Vaughan DE. Angiotensin and vascular fibrinolytic balance. Am J Hypertens. 2002;15(1 Pt 2):3S-8S.

256. Huber D, Cramer EM, Kaufmann JE, Meda P, Masse JM, Kruithof EK, et al. Tissue-type plasminogen activator (t-PA) is stored in Weibel-Palade bodies in human endothelial cells both in vitro and in vivo. Blood. 2002;99(10):3637-45.

257. van der Poll T, de Jonge E, Levi M. Regulatory role of cytokines in disseminated intravascular coagulation. Semin Thromb Hemost. 2001;27(6):639-51.

258. Thogersen AM, Jansson JH, Boman K, Nilsson TK, Weinehall L, Huhtasaari F, et al. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. Circulation. 1998;98(21):2241-7.

259. Chu AJ. Tissue factor upregulation drives a thrombosis-inflammation circuit in relation to cardiovascular complications. Cell Biochem Funct. 2006;24(2):173-92.

260. Coughlin SR. Thrombin signalling and protease-activated receptors. Nature. 2000;407(6801):258-64.

261. de Jonge E, Friederich PW, Vlasuk GP, Rote WE, Vroom MB, Levi M, et al. Activation of coagulation by administration of recombinant factor VIIa elicits interleukin 6 (IL-6) and IL-8 release in healthy human subjects. Clin Diagn Lab Immunol. 2003;10(3):495-7.

262. Shebuski RJ, Kilgore KS. Role of inflammatory mediators in thrombogenesis. J Pharmacol Exp Ther. 2002;300(3):729-35.

263. Levi M, van der Poll T, Schultz M. Infection and inflammation as risk factors for thrombosis and atherosclerosis. Semin Thromb Hemost. 2012;38(5):506-14.

264. Kahan T, Eliasson K. The influence of long-term ACE inhibitor treatment on circulatory responses to stress in human hypertension. Am J Hypertens. 1999;12(12 Pt 1-2):1188-94.

265. Duggan J, Nussberger J, Kilfeather S, O'Malley K. Aging and human hormonal and pressor responsiveness to angiotensin II infusion with simultaneous measurement of exogenous and endogenous angiotensin II. Am J Hypertens. 1993;6(8):641-7.

266. Hemker HC, Al Dieri R, De Smedt E, Beguin S. Thrombin generation, a function test of the haemostatic-thrombotic system. Thromb Haemost. 2006;96(5):553-61.

267. Agren A, Wiman B, Schulman S. Laboratory evidence of hyperfibrinolysis in association with low plasminogen activator inhibitor type 1 activity. Blood Coagul Fibrinolysis. 2007;18(7):657-60.

268. Fogari R, Zoppi A, Lazzari P, Preti P, Mugellini A, Corradi L, et al. ACE inhibition but not angiotensin II antagonism reduces plasma fibrinogen and insulin resistance in overweight hypertensive patients. J Cardiovasc Pharmacol. 1998;32(4):616-20.

269. Luc G, Bard JM, Juhan-Vague I, Ferrieres J, Evans A, Amouyel P, et al. C-reactive protein, interleukin-6, and fibrinogen as predictors of coronary heart disease: the PRIME Study. Arterioscler Thromb Vasc Biol. 2003;23(7):1255-61.

270. Kannel WB, Wolf PA, Castelli WP, D'Agostino RB. Fibrinogen and risk of cardiovascular disease. The Framingham Study. JAMA. 1987;258(9):1183-6.

271. Levenson J, Giral P, Razavian M, Gariepy J, Simon A. Fibrinogen and silent atherosclerosis in subjects with cardiovascular risk factors. Arterioscler Thromb Vasc Biol. 1995;15(9):1263-8.

272. Tosetto A, Prati P, Baracchini C, Manara R, Rodeghiero F. Association of plasma fibrinogen, C-reactive protein and G-455>A polymorphism with early atherosclerosis in the VITA Project cohort. Thromb Haemost. 2011;105(2):329-35.

273. Wu KK, Folsom AR, Heiss G, Davis CE, Conlan MG, Barnes R. Association of coagulation factors and inhibitors with carotid artery atherosclerosis. Early results of the Atherosclerosis Risk in Communities (ARIC) Study. Ann Epidemiol. 1992;2(4):471-80.

274. Tsakadze NL, Zhao Z, D'Souza SE. Interactions of intercellular adhesion molecule-1 with fibrinogen. Trends Cardiovasc Med. 2002;12(3):101-8.

275. Felmeden DC, Spencer CG, Chung NA, Belgore FM, Blann AD, Beevers DG, et al. Relation of thrombogenesis in systemic hypertension to angiogenesis and endothelial damage/dysfunction (a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial [ASCOT]). Am J Cardiol. 2003;92(4):400-5.

276. Burger D, Montezano AC, Nishigaki N, He Y, Carter A, Touyz RM. Endothelial microparticle formation by angiotensin II is mediated via Ang II receptor type I/NADPH oxidase/ Rho kinase pathways targeted to lipid rafts. Arterioscler Thromb Vasc Biol. 2011;31(8):1898-907.

277. Cordazzo C, Neri T, Petrini S, Lombardi S, Balia C, Cianchetti S, et al. Angiotensin II induces the generation of procoagulant microparticles by human mononuclear cells via an angiotensin type 2 receptor-mediated pathway. Thromb Res. 2013;131(4):e168-74.

278. Soejima H, Ogawa H, Yasue H, Kaikita K, Takazoe K, Nishiyama K, et al. Angiotensin-converting enzyme inhibition reduces monocyte chemoattractant protein-1 and tissue factor levels in patients with myocardial infarction. J Am Coll Cardiol. 1999;34(4):983-8.

279. Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. N Engl J Med. 2000;342(3):145-53.

280. Sleight P, Yusuf S, Pogue J, Tsuyuki R, Diaz R, Probstfield J. Blood-pressure reduction and cardiovascular risk in HOPE study. Lancet. 2001;358(9299):2130-1.

281. Duarte RCF, Ferreira CN, Rios DRA, Reis HJd, Carvalho MdG. Thrombin generation assays for global evaluation of the hemostatic system: perspectives and limitations. Revista Brasileira de Hematologia e Hemoterapia. 2017.

282. Brown NJ, Gainer JV, Murphey LJ, Vaughan DE. Bradykinin stimulates tissue plasminogen activator release from human forearm vasculature through B(2) receptor-dependent, NO synthase-independent, and cyclooxygenase-independent pathway. Circulation. 2000;102(18):2190-6.

283. Cruden NL, Newby DE. Clots, kinins and coronaries. Atherosclerosis. 2005;183(2):189-98.

284. Gleerup G, Winther K. Decreased fibrinolytic activity and increased platelet function in hypertension. Possible influence of calcium antagonism. Am J Hypertens. 1991;4(2 Pt 2):168s-71s.

285. Landin K, Tengborn L, Smith U. Elevated fibrinogen and plasminogen activator inhibitor (PAI-1) in hypertension are related to metabolic risk factors for cardiovascular disease. J Intern Med. 1990;227(4):273-8.

286. Jansson JH, Johansson B, Boman K, Nilsson TK. Hypo-fibrinolysis in patients with hypertension and elevated cholesterol. J Intern Med. 1991;229(4):309-16.

287. Juhan-Vague I, Alessi MC, Vague P. Increased plasma plasminogen activator inhibitor 1 levels. A possible link between insulin resistance and atherothrombosis. Diabetologia. 1991;34(7):457-62.

288. Cubeddu LX, Pool JL, Bloomfield R, Klotman PE, Pickering BI, Wombolt DG, et al. Effect of doxazosin monotherapy on blood pressure and plasma lipids in patients with essential hypertension. Am J Hypertens. 1988;1(2):158-67.

289. Shieh SM, Sheu WH, Shen DC, Fuh MM, Chen YD, Reaven GM. Glucose, insulin, and lipid metabolism in doxazosin-treated patients with hypertension. Am J Hypertens. 1992;5(11):827-31.

290. Fuh MM, Shieh SM, Wu DA, Chen YD, Reaven GM. Abnormalities of carbohydrate and lipid metabolism in patients with hypertension. Arch Intern Med. 1987;147(6):1035-8.

291. Jeng JR, Sheu WH, Jeng CY, Huang SH, Shieh SM. Effect of doxazosin on fibrinolysis in hypertensive patients with and without insulin resistance. Am Heart J. 1996;132(4):783-9.

292. Mihara M, Hashizume M, Yoshida H, Suzuki M, Shiina M. IL-6/IL-6 receptor system and its role in physiological and pathological conditions. Clin Sci (Lond). 2012;122(4):143-59.

293. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. Nat Immunol. 2015;16(5):448-57.

294. Rose-John S. IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. Int J Biol Sci. 2012;8(9):1237-47.

295. Hoch RC, Schraufstatter IU, Cochrane CG. In vivo, in vitro, and molecular aspects of interleukin-8 and the interleukin-8 receptors. J Lab Clin Med. 1996;128(2):134-45.

296. Boekholdt SM, Peters RJ, Hack CE, Day NE, Luben R, Bingham SA, et al. IL-8 plasma concentrations and the risk of future coronary artery disease in apparently healthy men and women: the EPIC-Norfolk prospective population study. Arterioscler Thromb Vasc Biol. 2004;24(8):1503-8.

297. Bhagat K, Vallance P. Inflammatory cytokines impair endothelium-dependent dilatation in human veins in vivo. Circulation. 1997;96(9):3042-7.

298. Zinman B, Hanley AJ, Harris SB, Kwan J, Fantus IG. Circulating tumor necrosis factor-alpha concentrations in a native Canadian population with high rates of type 2 diabetes mellitus. J Clin Endocrinol Metab. 1999;84(1):272-8.

299. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest. 1995;95(5):2409-15.

300. Togashi N, Ura N, Higashiura K, Murakami H, Shimamoto K. Effect of TNF-alpha--converting enzyme inhibitor on insulin resistance in fructose-fed rats. Hypertension. 2002;39(2 Pt 2):578-80.

301. Dawson TC, Kuziel WA, Osahar TA, Maeda N. Absence of CC chemokine receptor-2 reduces atherosclerosis in apolipoprotein E-deficient mice. Atherosclerosis. 1999;143(1):205-11.

302. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res. 2009;29(6):313-26.

303. Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griendling KK, et al. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. J Clin Invest. 1996;97(8):1916-23.

304. Grafe M, Auch-Schwelk W, Zakrzewicz A, Regitz-Zagrosek V, Bartsch P, Graf K, et al. Angiotensin II-induced leukocyte adhesion on human coronary endothelial cells is mediated by E-selectin. Circ Res. 1997;81(5):804-11.

305. Jilma B, Li-Saw-Hee FL, Wagner OF, Beevers DG, Lip GY. Effects of enalapril and losartan on circulating adhesion molecules and monocyte chemotactic protein-1. Clin Sci (Lond). 2002;103(2):131-6.

306. Kintscher U, Kon D, Wakino S, Goetze S, Graf K, Fleck E, et al. Doxazosin inhibits monocyte chemotactic protein 1-directed migration of human monocytes. J Cardiovasc Pharmacol. 2001;37(5):532-9.

307. Jekell A, Malmqvist K, Wallen NH, Mortsell D, Kahan T. Markers of inflammation, endothelial activation, and arterial stiffness in hypertensive heart disease and the effects of treatment: results from the SILVHIA study. J Cardiovasc Pharmacol. 2013;62(6):559-66.

308. Derosa G, Cicero AF, D'Angelo A, Tinelli C, Ciccarelli L, Piccinni MN, et al. Effect of doxazosin on C-reactive protein plasma levels and on nitric oxide in patients with hypertension. J Cardiovasc Pharmacol. 2006;47(4):508-12.

309. Calo LA, Bertipaglia L, Pagnin E, Davis PA, Sartori M, Semplicini A, et al. Effect of doxazosin on oxidative stress related proteins in essential hypertensive patients. Clin Exp Hypertens. 2006;28(2):181-8.

310. Yudkin JS, Juhan-Vague I, Hawe E, Humphries SE, di Minno G, Margaglione M, et al. Low-grade inflammation may play a role in the etiology of the metabolic syndrome in patients with coronary heart disease: the HIFMECH study. Metabolism. 2004;53(7):852-7.

311. Mertens I, Verrijken A, Michiels JJ, Van der Planken M, Ruige JB, Van Gaal LF. Among inflammation and coagulation markers, PAI-1 is a true component of the metabolic syndrome. Int J Obes (Lond). 2006;30(8):1308-14.

312. Veerkamp MJ, de Graaf J, Stalenhoef AF. Role of insulin resistance in familial combined hyperlipidaemia. Arterioscler Thromb Vasc Biol. 2005;25(5):1026-31.

313. Karasek D, Vaverkova H, Halenka M, Slavik L, Novotny D. Endothelial haemostatic markers in members of families with familial combined hyperlipidaemia. Thromb Res. 2009;123(3):466-75.

314. Hutley L, Prins JB. Fat as an endocrine organ: relationship to the metabolic syndrome. Am J Med Sci. 2005;330(6):280-9.

315. Madjid M, Awan I, Willerson JT, Casscells SW. Leukocyte count and coronary heart disease: implications for risk assessment. J Am Coll Cardiol. 2004;44(10):1945-56.

316. Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. Circulation. 2000;102(18):2165-8.

317. Rifai N, Ridker PM. High-sensitivity C-reactive protein: a novel and promising marker of coronary heart disease. Clin Chem. 2001;47(3):403-11.

318. Huang ZS, Chien KL, Yang CY, Tsai KS, Wang CH. Peripheral differential leukocyte counts in humans vary with hyperlipidaemia, smoking, and body mass index. Lipids. 2001;36(3):237-45.

319. Fruchart JC, Duriez P. HDL and triglyceride as therapeutic targets. Curr Opin Lipidol. 2002;13(6):605-16.

320. Pedersen OD, Gram J, Jespersen J. Plasminogen activator inhibitor type-1 determines plasmin formation in patients with ischaemic heart disease. Thromb Haemost. 1995;73(5):835-40.

321. Agewall S, Wikstrand J, Fagerberg B. Prothrombin fragment 1+2 is a risk factor for myocardial infarction in treated hypertensive men. J Hypertens. 1998;16(4):537-41.

322. Ten Cate H, Hemker HC. Thrombin Generation and Atherothrombosis: What Does the Evidence Indicate? J Am Heart Assoc. 2016;5(8).

323. Kapiotis S, Jilma B, Quehenberger P, Ruzicka K, Handler S, Speiser W. Morning hypercoagulability and hypofibrinolysis. Diurnal variations in circulating activated factor VII, prothrombin fragment F1+2, and plasmin-plasmin inhibitor complex. Circulation. 1997;96(1):19-21.

324. Ekholm M, Kahan T, Jorneskog G, Broijersen A, Wallen NH. Infusion of angiotensin II increases fibrinolysis in healthy individuals but not in patients with familial combined hyperlipidaemia. Blood Coagul Fibrinolysis. 2016;27(1):113-6.

325. Al-Merani SA, Brooks DP, Chapman BJ, Munday KA. The half-lives of angiotensin II, angiotensin II-amide, angiotensin III, Sar1-Ala8-angiotensin II and renin in the circulatory system of the rat. J Physiol. 1978;278:471-90.

326. Biasucci LM, Liuzzo G, Caligiuri G, Quaranta G, Andreotti F, Sperti G, et al. Temporal relation between ischemic episodes and activation of the coagulation system in unstable angina. Circulation. 1996;93(12):2121-7.

327. Jafri SM, VanRollins M, Ozawa T, Mammen EF, Goldberg AD, Goldstein S. Circadian variation in platelet function in healthy volunteers. Am J Cardiol. 1992;69(9):951-4.

328. Biemond BJ, Levi M, Ten Cate H, Van der Poll T, Buller HR, Hack CE, et al. Plasminogen activator and plasminogen activator inhibitor I release during experimental endotoxaemia in chimpanzees: effect of interventions in the cytokine and coagulation cascades. Clin Sci (Lond). 1995;88(5):587-94.

329. Eliasson M, Evrin PE, Lundblad D, Asplund K, Rånby M. Influence of gender, age and sampling time on plasma fibrinolytic variables and fibrinogen. Fibrinolysis. 1993;7(5):316-23.

330. Andreotti F, Kluft C. Circadian variation of fibrinolytic activity in blood. Chronobiol Int. 1991;8(5):336-51.

331. Mehta JL, Li DY, Yang H, Raizada MK. Angiotensin II and IV stimulate expression and release of plasminogen activator inhibitor-1 in cultured human coronary artery endothelial cells. J Cardiovasc Pharmacol. 2002;39(6):789-94.

332. Skurk T, Lee YM, Hauner H. Angiotensin II and its metabolites stimulate PAI-1 protein release from human adipocytes in primary culture. Hypertension. 2001;37(5):1336-40.

333. Luther JM, Gainer JV, Murphey LJ, Yu C, Vaughan DE, Morrow JD, et al. Angiotensin II induces interleukin-6 in humans through a mineralocorticoid receptor-dependent mechanism. Hypertension. 2006;48(6):1050-7.

334. Krejcy K, Eichler HG, Jilma B, Kapiotis S, Wolzt M, Zanaschka G, et al. Influence of angiotensin II on circulating adhesion molecules and blood leukocyte count in vivo. Can J Physiol Pharmacol. 1996;74(1):9-14.

335. Lottermoser K, Ulrich-Merzenich G, Vetter H, Dusing R. [Effects of angiotensin II on inflammation mediators in healthy subjects]. Dtsch Med Wochenschr. 2003;128(47):2470-5.

336. Ruiz-Ortega M, Ruperez M, Lorenzo O, Esteban V, Blanco J, Mezzano S, et al. Angiotensin II regulates the synthesis of proinflammatory cytokines and chemokines in the kidney. Kidney Int Suppl. 2002(82):S12-22.

337. Alessandri C, Basili S, Maurelli M, Bracaglia D, Andreozzi P, Pergolini M, et al. Relationship between prothrombin activation fragment F1 + 2 and serum cholesterol. Haemostasis. 1996;26(4):214-9.

338. Jay RH, Rampling MW, Betteridge DJ. Abnormalities of blood rheology in familial hypercholesterolaemia: effects of treatment. Atherosclerosis. 1990;85(2-3):249-56.

339. Otto C, Ritter MM, Richter WO, Minkenberg R, Schwandt P. Hemorrheologic abnormalities in defined primary dyslipoproteinemias with both high and low atherosclerotic risks. Metabolism. 2001;50(2):166-70.

340. Ekholm M, Kahan T, Jorneskog G, Broijersen A, Wallen NH. Angiotensin II infusion in man is proinflammatory but has no short-term effects on thrombin generation in vivo. Thromb Res. 2009;124(1):110-5.

341. Smid M, Dielis AW, Winkens M, Spronk HM, van Oerle R, Hamulyak K, et al. Thrombin generation in patients with a first acute myocardial infarction. J Thromb Haemost. 2011;9(3):450-6.

342. Skeppholm M, Kallner A, Malmqvist K, Blomback M, Wallen H. Is fibrin formation and thrombin generation increased during and after an acute coronary syndrome? Thromb Res. 2011;128(5):483-9.